276

4897

D-4992

276

Texas Tech University Health Sciences Center
SCHOOL OF MEDICINE / Department of Preventive Medicine and Community Health
National Pesticide Telecommunication Network (NPTN)
Lubbock, Texas 79430
(806) 743-3098 1-800-858-7378

September 7, 1988

Ben Nguyen
Cartwright, Slobodin, et al, Counselors at Law
101 California Street, Suite 2600
San Francisco, California 94111

Dear Mr. Nguyen:

I am enclosing copies of labels for Esteron 99, Formula 40, and Esteron 245. I am also including information on 2,4-D, 2,4,5-T and Sivex which are the chemicals in these. The contents of each herbicide you listed is below:

Esteron 245 - 2,4,5-T
Kuron - Silvex
Formula 40 - 2,4-D
2,4-Dow Weed Killer (I could not find this product)
Esteron 99 - 2,4-D

We do not have some of the information you requested. I think Freedom of Information would be your best source or the Registration Division of the Environmental Protection Agency. The addresses are below.

Freedom of Information
FOI Officer (A-101)
USEPA
401 M Street, SW
Washington, DC 20460

Richard Mountfort
Registration Division (TS-767C)
USEPA
401 M Street, SW
Washington, DC 20460

Thank you for writing and let me know if I can be of any further help.

Sincerely,

Ronadin Carey

Ronadin Carey (Ms.)
Pesticide Specialist

4898
D-4993

The LD_{50} and How To Interpret It

Pesticide toxicologists use rather simple animal toxicity tests to rank pesticides according to their toxicity. Long before pesticides are registered with the Environmental Protection Agency and eventually released for public use, the manufacturer must delcare the toxicity of their pesticide to the white rat under laboratory conditions. This toxicity is defined by the LD_{50} expressed as milligrams (mg) of toxicant per kilogram (kg) of body weight, the dose that kills 50 percent of the test animals to which it is administered under experimental conditions.

The LD_{50} is measured in terms of oral (fed to, or placed directly in the stomachs of rats), dermal (applied to the skin of rats or rabbits), and respiratory toxicity (inhaled).

Combined tabulation of pesticide toxicity classes.

	Routes of absorption		
Toxicity rating	LD_{50} Single oral dose for rats, mg/kg	LD_{50} Single dermal dose for rabbits mg/kg	Probable lethal oral dose for man
6—Supertoxic	< 5	< 20	A taste, a grain
5—Extremely toxic	5-50	20-200	A pinch, 1 teaspoon
4—Very toxic	50-500	200-1,000	1 teaspoon to 2 tablespoons
3—Moderately toxic	500-5,000	1,000-2,000	1 ounce to 1 pint
2—Slightly toxic	5,000-15,000	2,000-20,000	1 pint to 1 quart
1—Practically nontoxic	> 15,000	> 20,000	> 1 quart

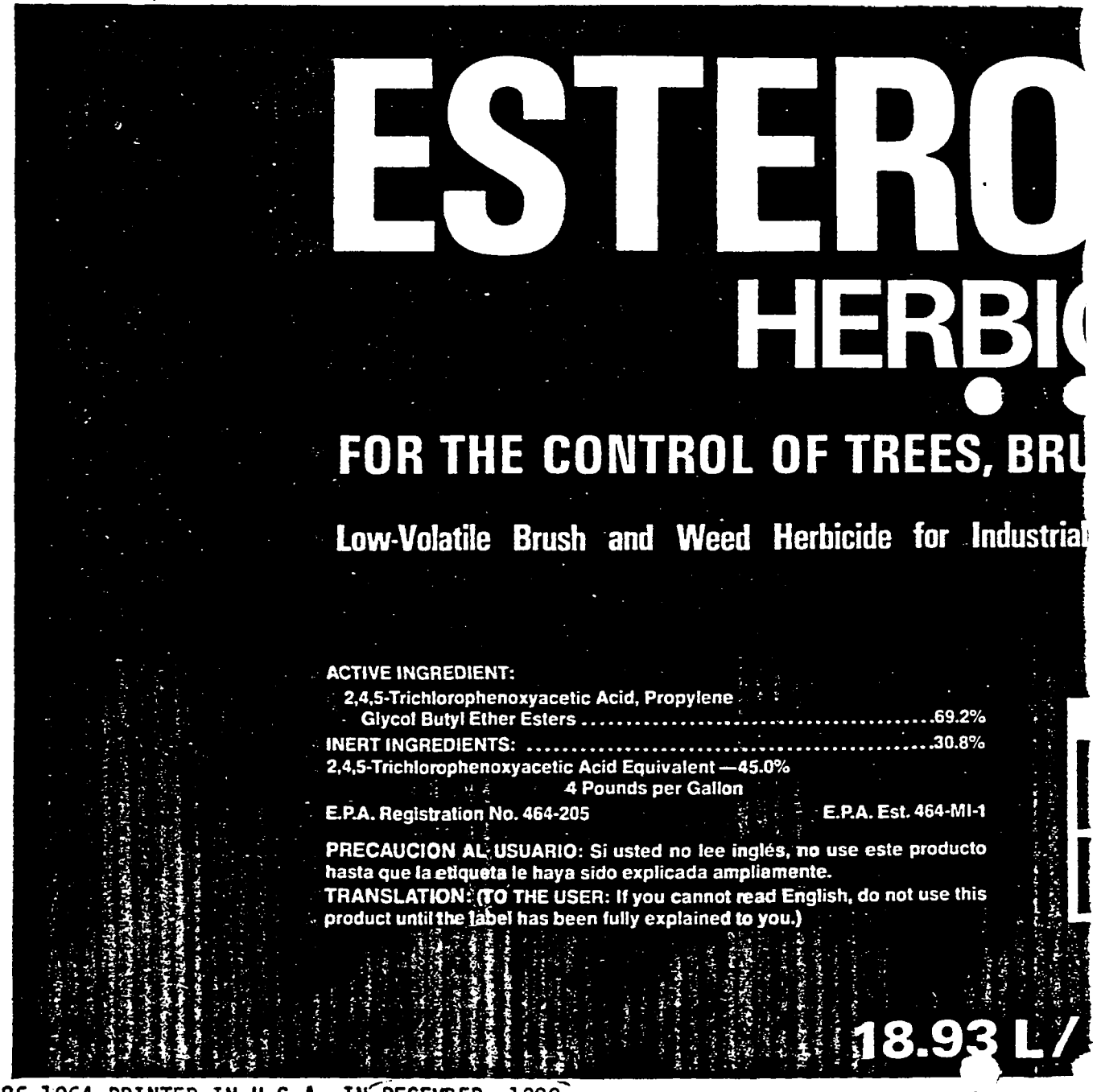
Source: Toxicity ratings modified from M. N. Gleason, R. E. Gleason, and H. C. Hodge. 1976. *Clinical Toxicology of Commercial Products.* 4th ed. Williams and Wilkins Company, Baltimore, Md. p.6

* In LD_{50} tests, 100% concentrates of the chemical are used (unless otherwise stated), whereas when you use the chemical, it is diluted to a lower concentration which has a lower toxicity.
Example: Carbaryl
LD_{50} = 500 mg/kg = Moderately to Very toxic
5% Sevin Dust → LD_{50} = 10,000 mg/kg = Slightly toxic
(Carbaryl is active ingredient)

The Pesticide Book
George W. Ware, W. H. Freeman & Co.
San Francisco - 1978

4899

D-4994



86-1064 PRINTED IN U.S.A. IN DECEMBER, 1980.
REPLACES SPECIMEN LABEL 86-1064 PRINTED IN JANUARY, 1980.
DISCARD PREVIOUS SPECIMEN LABELS.
REVISIONS INCLUDE: REPRINTED TO CORRECT ERROR IN PRECAUTIONS;
CONTAINER GRAPHICS WERE CORRECT; SPECIMEN LABEL OF JANUARY 1980

4900
D-4995

DOW*

SPECIMEN LABEL
REDUCED TO 75%

RON* 245
RBICIDE

EES, BRUSH AND BROADLEAF WEEDS

for Industrial Vegetation Control, Fencerows, and Rangeland

...69.2%
...30.8%

464-MI-1

producto

t use this

KEEP OUT OF THE REACH OF CHILDREN

CAUTION

MAY BE HARMFUL IF SWALLOWED • MAY CAUSE IRRITATION
Avoid Contact with Eyes, Skin and Clothing
Do Not Cut or Weld Container

In case of an emergency endangering life or property involving this product, call collect
517-636-4400

AGRICULTURAL CHEMICAL
Do Not Ship or Store with Food, Feeds, Drugs or Clothing

3 L/5 gal

4901
D-4996

RON 245 HERBICIDE

ıer Esters of 2,4,5-T • Acid Equivalent: 4 Pounds per Gallon

gallons of ESTERON 245 in 100 gallons of oil. Brush of average) feet high may take up to 150 gallons of spray mixture per acre.

nt: Where growth is more than 6 to 8 feet tall, cut it close to the the freshly cut stumps and stubs with 3 gallons of ESTERON (1 pint in 4 gallons) of oil, mixed thoroughly. For more resistant llons of ESTERON 245 in 100 gallons (1 pint in 3 gallons) of oil. ll exposed bark, as well as cut surfaces. This means spraying un-n-off to the ground line is noticeable. Old or rough bark requires ne than young or smooth bark. Apply at any time, including :cept when ice, snow or water prevent spraying to the ground are obtained on freshly cut stumps two inches across or larger. ge normally requires from 10 to 100 gallons per acre depending nps and stubs.

it: For large trees, make a singlehack girdle or "frill" of over-completely around the tree as close to the ground as feasible. roughly using a mixture of 2 gallons of ESTERON 245 in 100 n 3 gallons) of oil.

atment: Use 1/4 pint of ESTERON 245 in 3 gallons of water all foliage, shoots, stems and bark without runoff.

LOW VOLUME SPRAYS

ne sprays containing ESTERON 245 when foliage is well nts are actively growing. For best results on woody species, soil e sufficient to promote foliar growth. Spraying during prolong-er or after leaves have lost their normal green color and vigor sfactory control. Apply low volume sprays by air or ground hen spray drift will not be a problem – note use precautions.

Usi wered Knapsack Sprayer—Mix 1 1/2 to 2 gallons with) or kerosene to make 20 gallons of total spray solu-portab. apsack mistblower to all sides of lower brush stems collar. Good coverage of the root collar is essential for best blower at 1/4 to 1/3 throttle for best spray delivery and ximum drift control use a basal nozzle attachment and do not the horizontal position.

APPLICATION FOR BRUSH CONTROL

ltural Experiment Station, your local Extension Service Weed ts for best time to treat and need for re-treatment in your area. arly boot to milk stage where grass seed production is desired.

int ESTERON 245 plus 1/2 to 1 gallon of oil in enough water to total spray per acre. Apply 40 to 90 days after first leaves

ak: Use 1/2 to 1 quart of ESTERON 245 plus 1 gallon of oil in nake 4 gallons of total spray per acre.

ck Oaks: Use 2 quarts of ESTERON 245 plus 1 gallon of oil in nake 4 to 6 gallons of total spray per acre.

USE PRECAUTIONS

e dairy animals on treated areas within 6 weeks after applica-meat animals on treated areas within 2 weeks of slaughter.

T WITH 2,4,5-T SUSCEPTIBLE CROPS AND OTHER ADLEAF PLANTS—ESTERON 245 Herbicide is injurious to nts. Therefore, do not apply directly to or otherwise permit nts to contact cotton, grapes, tobacco, fruit trees, vegetables, ls or other desirable plants susceptible to 2,4,5-T. Do not use ouse.

IN THE VICINITY OF COTTON, GRAPES, TOBACCO, OTHER DESIRABLE 2,4,5-T SUSCEPTIBLE CROPS OR LANTS.

DO NOT SPRAY WHEN WIND IS BLOWING TOWARDS SUSCEPTIBLE CROPS OR ORNAMENTAL PLANTS.

AVOID SPRAY DRIFT—Applications should be made only when there is no hazard from spray drift since very small quantities of spray, which may not be visible, may severely injure susceptible crops during both growing and dormant periods. Use coarse sprays to minimize drift since, under adverse weather conditions, fine spray droplets may drift a mile or more. The spray thickening agent, NALCO-TROL[1], may be used with this product to aid in reducing spray drift. If used follow all use recommendations and precautions on the product label.

[1]NALCO-TROL – Trademark of NALCO Chemical Company

GROUND EQUIPMENT—With ground equipment, spray drift can be lessened by keeping the spray boom as low as possible; by applying 20 gallons or more of spray per acre; by using no more than 20 pounds spraying pressure with large droplet producing nozzle tips; by spraying when wind velocity is 8 miles per hour or less. Do not apply with hollow cone-type insecticide or other nozzles that produce a fine-droplet spray.

AERIAL APPLICATION—With aircraft, drift can be lessened by applying a coarse spray; by using no more than 20 pounds spray pressure at the nozzles; by using straight stream nozzles directed straight back; by using a spray boom no longer than 3/4 the wing span of the aircraft; and by spraying only when wind velocity is less than 6 mph.

DO NOT APPLY BY AIRCRAFT WHEN AN AIR TEMPERATURE INVERSION EXISTS. Such a condition is characterized by little or no wind and with air temperature lower near the ground than at higher levels. The use of a continuous smoke column at or near site of application is suggested to indicate direction and velocity of air movement, and to indicate a temperature inversion by layering of the smoke.

At high temperatures (above 95°F) vapors from this product may injure susceptible plants growing nearby. Do not use in or near a greenhouse. Excessive amounts of this herbicide in the soil may temporarily inhibit seed germination or plant growth.

Do not use around the home, recreation areas or similar sites.

This product is toxic to fish. Keep out of lakes, streams, and ponds. Do not contaminate water by cleaning of equipment or disposal of wastes.

Do not contaminate irrigation ditches or water used for irrigation or domestic purposes. This product can be stored in an unheated building but if exposed to subfreezing temperatures, should be warmed to at least 40°F and mixed thoroughly before using. Do not store near fertilizers, seeds, insecticides or fungicides. Do not reuse containers. To avoid injury to desirable plants, do not store, handle or apply other agricultural chemicals with the same containers or equipment used with ESTERON 245 except as specified on this label.

Rinse equipment and containers and dispose of waste by burying in non-crop lands away from water supplies. Containers should be disposed by punching holes in them and burying with waste or follow official local recommendations for container disposal.

Local conditions may affect the use of herbicides. Consult your State Agricultural Experiment Station or Extension Service weed specialist for advice in selecting treatments from this label to best fit local conditions. Be sure that use of this product conforms to all applicable regulations. Apply this product only as specified on this label.

NOTICE: Seller warrants that the product conforms to its chemical description and is reasonably fit for the purpose stated on the label when used in accordance with directions under normal conditions of use, but neither this warranty nor any other warranty of MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, express or implied, extends to the use of this product contrary to label instructions, or under abnormal conditions, or under conditions not reasonably foreseeable to seller, and buyer assumes the risk of any such use

10586-049-9 U1280

CHEMICAL COMPANY
AND SUBSIDIARIES
364 SA HORGEN, SWITZERLAND HONG KONG
RI 134, USA SARNIA, ONTARIO, CANADA
HE DOW CHEMICAL COMPANY

4902

D-4997

SPECIMEN LABEL
(BACK)
REDUCED TO 75%

DOW

ESTER(

Contains Propylene Glycol Butyl Ether Esters

DIRECTIONS FOR USE

ESTERON 245 herbicide is recommended for industrial vegetation control, fencerows, and rangeland. **Do not use in forest lands, rights-of-way, or pastures.**

This herbicide controls herbaceous and woody plants including such 2,4-D resistant species as—ash, black gum, brambles, groundcherry, hawthorn, horsenettle, maple, mesquite, oak, osageorange, palmetto, poison ivy, pricklypear cactus, redbay, salmonberry, sweetgum, wild blackberry, wild rose, and certain species of Ribes. **Do not apply ESTERON 245 where spray drift may contact nearby 2,4,5-T susceptible crops or other desirable plants or may contaminate water intended for irrigation or domestic purposes. Read and follow all Use Precautions given on this label.**

PREPARING THE SPRAY

Use only diesel oil, No. 1 or No. 2 fuel oil or kerosene where oil is recommended in the spray mixture.

Oil sprays: Add ESTERON 245 to the required amount of oil in the spray tank or mixing tank and mix thoroughly. This mixture can be made at any time before actual use and no separation will occur. Do not let any water, or oil-water mixture sprays get into the ESTERON 245 or into the finished mixture, as it may form a gel.

Water Sprays: Fill the spray tank about half full with clean water, add the required amount of ESTERON 245 and complete filling the tank. Mix thoroughly and continue agitation while spraying. **Caution: See NOTE in paragraph on Oil-Water Mixture Sprays.**

Oil-Water Mixture Sprays: When vigorous agitation is used, 1 gallon of ESTERON 245 will emulsify up to 10 gallons of oil in 100 gallons of spray mixture. First, premix the ESTERON 245 and oil in a separate container. Do not allow any water or mixtures containing water to get into the ESTERON 245 or the premix. Fill the spray tank about half full with water, then slowly add the premix with continuous agitation and complete filling the tank with water. If the premix is put in the tank without any water, the first water added may form a thick "invert" (water in oil) emulsion which will be hard to break. As an alternate procedure, the oil may be added after the ESTERON 245 is mixed in the water; but highly vigorous mechanical agitation is required and a poor emulsion may be formed. The premix method is preferred.

NOTE: ESTERON 245 in water or oil-water sprays forms an emulsion, not a solution, and separation may take place unless sprays are agitated continously. Mechanical agitation is recommended.

HIGH VOLUME SPRAYS

Basal Bark Treatment: Brush and small trees can be controlled by spraying the basal parts of brush stems and tree trunks to a height of 12 to 15 inches from the ground line. Use a solution of 3 gallons of ESTERON 245 in 100 gallons (1 pint in 4 gallons) of oil. With certain resistant species, 4 gallons of ESTERON 245 in 100 gallons (1 pint in 3 gallons) of oil, is effective. As only the basal portions of the brush are treated on a spot basis, the total amount sprayed per acre would not be expected to exceed 100 gallons. Knapsack or power equipment may be used, but complete wetting of the indicated area is necessary, particularly at the ground line. This means spraying until run-down or run-off to the ground line is noticeable. Old or rough bark requires more spray than young or smooth bark. Low pressures are desirable. Apply at any time, including the winter months, except when snow, ice or water prevent spraying to the ground line. Often delayed response and killing can be expected.

Dormant Brush: Treat any time after brush is dormant and most of the foliage has dropped. Spray should be concentrated at the base of stems and in addition, the upper parts of the stems should be broadcast sprayed enough to wet them. Under rootsuckering species such as sumac, persimmon, sassafras and locust, also spray the ground area to control small root suckers that may not be readily visible. Mix 1 1/2 gallons of EST[illegible] density and 4 to 6 feet high may

Stump Treatment: Where grow ground and spray the freshly cu 245 in 100 gallons (1 pint in 4 gall species, use 4 gallons of ESTER Wet thoroughly all exposed bark, til run-down or run-off to the gro more spray volume than young winter months, except when ice line. Best results are obtained on Adequate coverage normally req on density of stumps and stubs.

"Frill" Treatment: For large tre lapping axe cuts completely arou Spray the frill thoroughly using gallons (1/2 pint in 3 gallons) of

Spot Foliage Treatment: Use and spray to wet all foliage, sho

LOW V

Apply low volume sprays con developed and plants are actively moisture should be sufficient to p ed hot, dry weather or after leav may not give satisfactory contr equipment only when spray drift

Basal Treatment Using Power of ESTER[illegible]45 with f[illegible] or tion. App[illegible]h a portab[illegible]aps including the root collar. Good results. Run mistblower at 1/4 coverage. For maximum drift cor raise nozzle above the horizontal

AIR APPLICATI(

Consult the Agricultural Experime or Range specialists for best time Do not use from early boot to mil

Mesquite: Use 1 pint ESTERON 2 make 4 gallons of total spray pe appear.

Sand Shinnery Oak: Use 1/2 to enough water to make 4 gallons

Post and Blackjack Oaks: Use enough water to make 4 to 6 gall

USE F

Note: Do not graze dairy animals tion. Do not graze meat animals

AVOID CONTACT WITH 2,4,5 DESIRABLE BROADLEAF PLA most broadleaf plants. Therefore, even minute amounts to contact flowers, ornamentals or other des in or near a greenhouse.

DO NOT APPLY IN THE VICI TOMATOES OR OTHER DESI ORNAMENTAL PLANTS.

THE DOW CHE
AND
MIDLAND, MICHIGAN 48640, USA
CORAL GABLES, FLORIDA 33
Trademark HE

4903

D-4998

DOW

MAY NOT BE PRODUCT OR FORMULATION USED IN YOUR AREA

MAY NOT BE PRODUCT OR FORMULATION USED IN YOUR AREA

Esteron Concen

low-v

CONTAINS BUTOXYETHYL

For the Control of Many Broadleaf Wee
and Woody Plants Susceptible to 2,4-D in Grass Past

ACTIVE INGREDIENT	
2,4-Dichlorophenoxyacetic acid, Butoxyethyl Ester†	62.5%
INERT INGREDIENTS	37.5%
2,4-Dichlorophenoxyacetic Acid Equivalent: 43.2%-3.8 lb/gal	

†Isomer Specific by AOAC Method No. 6.275-6.279 (13th Ed.)
EPA Reg. No. 464-566 EPA Est. 464-MI-1[MM]; 359-OR-1[SP]
Superscript used corresponds to letters in LOT number.

PRECAUCION AL USUARIO: Si usted no lee inglés, no use este producto hasta que la etiqueta le haya sido explicada ampliamente.
TRANSLATION: (TO THE USER: If you cannot read English, do not use this product until the label has been fully explained to you.)

18.93 L / 5 gal

HAR
Avoid Contact w

In case of contact, in
irritation persists. If s
sticking finger down t
unconscious person.

This product is toxic t
likely to occur. Do no
contaminate irrigatio

In case of an emerg
property involving
517-

86-1674 PRINTED IN U.S.A. IN SEPTEMBER, 1982.
REPLACES SPECIMEN LABEL 86-1674 PRINTED IN MAY, 1982.
DISCARD PREVIOUS SPECIMEN LABELS.
REVISIONS INCLUDE: (1) REVISED WEED LIST, "USE IN LIQUID NITROGEN FERTILIZER" SECTION, "WEED CONTROL IN SMALL GRAINS..." SECTION, USE PRECAUTIONS, AND STORAGE AND DISPOSAL INSTRUCTIONS (_) ADDED

4904

D-4999

SPECIMEN LABEL
REDUCED TO 75%

on 99 entrate

w-volatile herbicide

YETHYL ESTER OF 2,4-D

dle Weeds, Herbaceous Perennials
rass Pastures, Certain Crops and Non-Crop Areas.

KEEP OUT OF REACH OF CHILDREN

CAUTION

PRECAUTIONARY STATEMENTS
Hazards to Humans and Domestic Animals
HARMFUL IF SWALLOWED • MAY CAUSE IRRITATION
void Contact with Skin, Eyes, or Clothing • Wash Thoroughly After Handling
Statements of Practical Treatment
se of contact, immediately flush eyes or skin with plenty of water. Get medical attention if ation persists. **If swallowed,** induce vomiting immediately by giving two glasses of water and ing finger down throat. Call a physician. Do not induce vomiting or give anything by mouth to an nscious person.
Physical or Chemical Hazards
Do Not Cut or Weld Container
Environmental Hazards
product is toxic to fish. Do not apply directly to any body of water. Do not apply where runoff is to occur. Do not contaminate water by cleaning of equipment or disposal of wastes. Do not aminate irrigation ditches or water used for irrigation or domestic purposes.

case of an emergency endangering life or operty involving this product, call collect:
517-636-4400

AGRICULTURAL CHEMICAL
Do Not Ship or Store with Foods, Feeds, Drugs or Clothing

4905

D-5000

SPECIMEN LABEL
(BACK)
REDUCED TO 75%

ugh or denting stage, apply 1 to 2 pints erennial weeds, decrease weed seed ed, cocklebur, dogbane, jimsonweed, with harvesting. Do not forage or feed

USE DRAIN FIELDS: Following the num, wild garlic and wild onion often 2 to 3 quarts per acre of ESTERON 99 part of a control program. Do not forage

per acre when sorghum is 5 to 15 inches to control some weeds but the chance il. Do not treat before the sorghum is 5 ugh stages. If sorghum is taller than 8 e as much as possible. Temporary crop re and high air temperatures. Varieties nsitive. Spray only varieties known to be ltural Experiment Station or Extension

e amount of water required for uniform lished stands in spring from the tiller to seedings may be treated with the lower al weed regrowth may be treated in the

GRASS PASTURES: NOTE: Do not graze ation. Do not use on bent grass, alfalfa, areas until grass is well established. Do d production is desired.

Shelder, Muskthistle and Other Broad- per acre in the amount of water needed growing actively. 1 quart per acre will al weeds may require repeated treat-

making three applications (fall-spring- ring.

grass: Apply 1 to 2 quarts per acre

use 1 quart in 5 gallons of oil or in 4 rcraft between May 15 and June 15. On d apply by aircraft when foliage is fully

acre in 2 to 3 gallons of oil or in 3 to 5 e 3 quart rate is usually required. Brush d. Retreatment may be needed.

tebrush and Certain Other Chaparral of water. One gallon of fuel oil may be Make applications by aircraft or ground ctive control, the brush must be fully ment may be needed.

co pecies susceptible to 2.4-D in e anks, spray brush up to 5 to 8 4 quarts of ESTERON 99 Concentrate including foliage, stems and bark. The quate coverage of solid stand of brush. the spray off the area being treated. ore frost as long as the soil moisture is ess effective in midsummer during hot are not actively growing. Oil or wetting ed effectiveness.

adleaf weeds and brush on sites to be STERON 99 Concentrate herbicide in y 6 to 25 gallons. Applications can be apsack sprayer). Two to eight quarts of ay be added to improve brush control.

nter or spring to control susceptible s, cascara, cherry, service cherry and reak, use ESTERON 99 Concentrate at r ground in sufficient spray volume for In plantations where pine or larch are

otible evergreen brush species, such as manzanita or deciduous brush after te at rates up to 3 quarts per acre alone ar oil or suggested rates of suitable STERON 99 Concentrate without oil. uarts per acre but may cause injury or e of spray for good coverage of brush. seriously injured by treatment at these

ne, jack pine, red pine, black spruce, d harden off and brush is still actively Concentrate per acre in enough water ground to control certain competing nd willow. Since this treatment may cannot be tolerated.

ne): Apply ESTERON 99 Concentrate eptible by directing spray around the units of spray. Rates of ESTERON 99 il-water, or water carrier at 10 to 100

VNS, GOLF COURSES, CEMETERIES, AINAGE DITCH BANKS: Apply 1 to 3 amount of water needed for uniform ed control under average conditions e on golf greens nor on dichondra or creeping grasses such as bent and until grass is well established nt. With spring application g ... jumes are usually damaged or Deep rooted perennial weeds may quent years.

ESTERON 99 Concentrate herbicide 2 1/2 gallons of water. Spray to wet all foliage (400-800 gallons per acre). Addition of a wetting agent may be advisable. Apply in the spring during flower head emergence. Respray if needed when regrowth is 3 to 5 feet tall.

WEED CONTROL ON FALLOW LAND: Use 1½ to 2¼ quarts per acre on annual broadleaf weeds and up to 3 quarts per acre on established perennial species, such as Canada thistle and field bindweed. Apply to actively growing weeds. Do not plant treated fallow land until three months after treatment, or until chemical has disappeared from soil.

SPOT TREATMENT: To control broadleaf weeds in small non-cropland areas with a hand sprayer, use ¼ pint of ESTERON 99 Concentrate in 3 gallons of water and spray to thoroughly wet all weed foliage. Keep spray mixture agitated to prevent separation.

CONTROL OF WOODY WEEDS IN LOW—BUSH BLUEBERRY FIELDS IN MAINE: How to use: Mount a drum 8 to 10 feet long or some other suitable length, and 1½ to 2 feet in diameter on an axle such as an old hay rake frame. Cover the drum with water absorbent yet tough cloth which will resist rapid wear and tear. Draw the cloth-covered drum across the blueberry field and at the same time spray evenly onto the full length of the top of the cloth-covered drum a spray mixture made by diluting 1 quart of ESTERON 99 Concentrate in 50 gallons of water per acre. Have the drum mounted so that as it revolves on its axis it is high enough to miss most of the low bush blueberry stems, yet low enough to forcibly brush the spray-saturated cloth-covered drum against the higher woody weeds, principally sweet fern, wild cherry and poplar. Keep the cloth wet enough to provide top coverage of the weeds, yet not so wet as to allow runoff of the *liquid which could cause injury to the blueberry plants.*

When to Use: Apply during June and July when weed tops have emerged sufficiently above the blueberry stems to allow treatment of the weeds and not the blueberry plants. Apply only during the year before the first burn. To use this method of weed control, two-year burns should be extended to three years. **Caution:** Do not allow the spray being applied to the cloth-covered drum to be directed onto the blueberries. Do not harvest-rake field during the herbicide treatment year or until a two-year interval thereafter.

USE PRECAUTIONS

AVOID CONTACT WITH 2,4-D SUSCEPTIBLE CROPS AND OTHER DESIRABLE BROADLEAF PLANTS: Do not apply directly to or otherwise permit even minute amounts to contact cotton, grapes, tobacco, fruit trees, vegetables, flowers, ornamentals or other desirable plants susceptible to 2,4-D. Do not use in or near a greenhouse.

DO NOT APPLY IN THE VICINITY OF COTTON, GRAPES, TOBACCO, TOMATOES OR OTHER DESIRABLE 2,4-D SUSCEPTIBLE CROPS OR PLANTS. DO NOT SPRAY WHEN WIND IS BLOWING TOWARDS SUSCEPTIBLE CROPS OR ORNAMENTAL PLANTS.

AVOID SPRAY DRIFT: Applications should be made only when there is no hazard from spray drift since very small quantities of spray, which may not be visible, may severely injure susceptible crops during both growing and dormant periods. Use coarse sprays to minimize drift since, under adverse weather conditions, fine spray droplets may drift a mile or more. A spray thickening agent such as NALCO TROL[1], may be used with this product to aid in reducing *spray drift. If used, follow all use recommendations and precautions on the product label.*

[1]NALCO TROL — Trademark of NALCO Chemical Company.

GROUND EQUIPMENT: With ground equipment, spray drift can be lessened by keeping the spray boom as low as possible; by applying 20 gallons or more of spray per acre; by using no more than 20 pounds spraying pressure at large droplet producing nozzle tips; by spraying when wind velocity is low; and by stopping all spraying when wind exceeds 6 to 7 miles per hour. Do not apply with hollow cone-type insecticide or other nozzles that produce a fine-droplet spray.

DETERMINE AIR MOVEMENT AND DIRECTIONS BEFORE FOLIAR APPLICATION: Use a smoke generator or other means at or near the application site for the detection of air movement, air stability or temperature inversions. Such a condition exists when there is little or no wind and air temperature is lower near the ground than at higher levels. Use appropriate drift control measures or avoid application when smoke is moving toward nearby desirable susceptible plants or sensitive areas.

AERIAL APPLICATION: With aircraft, drift can be lessened by applying a coarse spray; by using no more than 20 pounds spray pressure at the nozzles; by using straight stream nozzles directed straight back; by using a spray boom no longer that ¾ the wing or rotor span of the aircraft; and by spraying only when wind velocity is less than 6 mph.

Excessive amounts of this herbicide in the soil may temporarily inhibit seed germination or plant growth. Violent wind storms may move soil particles. If 2,4-D is on soil particles and they are blown onto the susceptible plants, visible symptoms may appear. Serious injury is unlikely. The hazard of movement of 2,4-D on dust is reduced if treated fields are irrigated or if rain occurs shortly after application.

AT HIGH TEMPERATURES, VAPORS FROM THIS PRODUCT MAY INJURE SUSCEPTIBLE PLANTS GROWING NEARBY.

To avoid injury to desirable plants, do not handle or apply other agricultural chemicals with the same equipment used for ESTERON 99 Concentrate unless appropriately cleaned first. Local conditions may affect the use of herbicides. Consult your State Agricultural Experiment Station or Extension Service weed specialists for cleaning methods which are in compliance with local regulations and for advice in selecting treatments from this label to best fit local conditions. Be sure that use of this product conforms to all applicable regulations. Apply this product only as specified on this label.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

STORAGE: Keep container tightly closed when not in use.

PESTICIDE DISPOSAL: Pesticide, spray mixture or rinsate that cannot be used according to label instructions must be disposed of according to Federal, State, or local procedures under the Resource Conservation and Recovery Act.

CONTAINER DISPOSAL: Triple rinse or equivalent and offer for recycling or reconditioning, or dispose of in a sanitary landfill, or by other approved State and local procedures.

MONEY BACK GUARANTEE

ESTERON 99 Concentrate herbicide is guaranteed by The Dow Chemical Company to the full extent of the purchase price:

1. **To give satisfactory control of weed and brush species listed on container when used as recommended.**
2. **To form a suitable spray mixture in any water fit for spray use.**
3. **To store satisfactorily at temperatures as low as −40°F.**

NOTICE: Seller warrants that the product conforms to its chemical description and is reasonably fit for the purposes stated on the label when used in accordance with the directions under normal conditions of use, but neither this warranty nor any other warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE, express or implied, extends to the use of this product contrary to label instructions, or under abnormal conditions, or under conditions not reasonably foreseeable to seller, and buyer assumes the risk of any such use.

10596-025-2 28682-B962

THE DOW CHEMICAL COMPANY
AND SUBSIDIARIES
MIDLAND, MICHIGAN 48640, USA HORGEN, SWITZERLAND HONG KONG
CORAL GABLES, FLORIDA 33134, USA SARNIA ONTARIO, CANADA

* Trademark of THE DOW CHEMICAL COMPANY

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WEED LIST

ESTERON 99 Concentrate herbicide is recommended for control of numerous broadleaf weeds and certain 2,4-D susceptible woody plants without injury to most established grasses. Species controlled include the following, plus many others:

beggarticks • bitterweed • blueweed, Texas • broomweed • buckbrush • buckwheat, wild burdock • burhead • carpetweed • catnip • chamise • chicory • cocklebur • coffeeweed cornflower • coyotebrush • croton • dandelion • docks • dogfennel • elderberry • gallnsoga garlic, wild • goatsbeard • halogeton • hemp, wild • jewelweed • jimsonweed • ladysthumb lambsquarter • loco, bigbend • mallow, Venice • manzanita • marshelder • milkvetch morningglory, annual • nettles • onion, wild • pennycress (fanweeed) • pepperweed, field pigweed†† • plantains • poorjoe • rabbitbrush • radish, wild • ragweed • rape, wild • redstem sage, coastalsagebrush, big • sagebrush, sand • salsify • sand shinnery oak shepherdspurse • sicklepod • smartweed (annual) • sneezeweed, bitter • sowthistle, annualspanishneedles • sumac • sunflower • sweetclover • tansyragwort • thistle, bull thistle, musk • thistle, Russian • tumbleweed • velvetleaf • vervains • vetch • water plantain wild mustard • willow • witchweed • wormwood • yellow rocket • yellow starthistle

††The control of "hybrid" pigweeds appears to be less satisfactory from 2,4-D products than formerly experienced on "non-hybrid" varieties. Since 2,4-D herbicides are not as effective on the "hybrid" pigweeds, it is necessary to apply higher rates of 2,4-D for control, especially later in the growing season. Higher rates injure some crops, so less than satisfactory pigweed control may be experienced by the highest tolerated crop dosages.

Therefore, The Dow Chemical Company no longer includes pigweed among the species covered by the performance guarantee statements on the labels for ESTERON 99 Concentrate herbicide. At this time, this disclaimer applies only to the High Plains of Texas and western Oklahoma, including the Panhandles. All other guarantees on these product labels are unchanged by this disclaimer.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

Apply ESTERON 99 Concentrate herbicide as water or oil spray during warm weather when weeds or brush are actively growing. Application under drought conditions often will give poor results. Use low spray pressure to minimize drift. On cropland and along roadsides, do not exceed 20 psi pressure. Apply enough spray volume to provide uniform coverage of weeds and brush, usually 5 to 20 gallons per acre by ground equipment and 3 to 5 gallons by aircraft. Higher gallonage may be used if desired to improve spray coverage. Generally, the lower dosages recommended on this label will be satisfactory for young, succulent growth of sensitive weed species. For less sensitive species and under conditions where control is more difficult, the higher dosages will be needed. For crop uses, do not mix with oil or other adjuvants unless specifically recommended on this label. Deep-rooted perennial weeds such as Canada thistle and field bindweed and many woody plants usually require repeated applications for maximum control. **Do not apply ESTERON 99 Concentrate where spray drift may contact nearby susceptible crops or other desirable plants or may contaminate water for irrigation or domestic use. Read and follow all Use Precautions given on this label.**

NOTE: If there are uncertainties concerning special local use situations or specific crop variety tolerances to 2,4-D, consult your State Agricultural Experiment Station or local Extension Service weed specialists for advice.

TO PREPARE THE SPRAY: (1) Fill the spray tank about half full with water, then add the required amount of ESTERON 99 Concentrate, with agitation, and finally the rest of the water. NOTE: ESTERON 99 Concentrate in water forms an emulsion which tends to separate unless the mixture is kept agitated. (2) If oil is added, first mix the ESTERON 99 Concentrate and the oil and then add this mixture to the water. However, with adequate agitation, the oil can be added after the ESTERON 99 Concentrate is mixed in the water. (3) If straight oil is used, a solution is formed and separation does not occur. Do not allow any water to get into the oil-herbicide mixture to avoid formation of an invert emulsion.

USE IN LIQUID NITROGEN FERTILIZER: ESTERON 99 Concentrate may be combined with liquid nitrogen fertilizer suitable for foliar application to accomplish weeding and feeding of corn, small grains or grass pastures in one operation. Use ESTERON 99 Concentrate in accordance with recommendations for these crops as given on this label. Use liquid fertilizer at rates recommended by supplier or Extension Service Specialist. Test for mixing compatibility using desired procedure and spray mix proportions in clear glass jar before mixing in spray tank. A compatibility aid such as UNITE™ OR COMPEX®† may be needed in some situations. Compatability is best with straight liquid nitrogen fertilizer solutions. Mixing with N-P-K solutions or suspensions may not be satisfactory, even with addition of a compatability aid. Pre-mixing ESTERON 99 Concentrate with 1 to 4 parts water may help in difficult situations.

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®Trademark of Kalo Laboratories

Fill the spray tank about half full with the liquid fertilizer, then add the ESTERON 99 Concentrate with agitation and complete filling the tank with fertilizer. Apply immediately and continue agitation in the spray tank during application. **Do not store the spray mixture.** Application during very cold weather (near freezing) is not advisable.

WEED CONTROL IN SMALL GRAINS NOT UNDERSEEDED WITH A LEGUME: NOTE: Do not permit dairy animals or meat animals being finished for slaughter to forage or graze treated grain fields within 2 weeks after treatment.

Wheat, Barley and Rye: Apply ½ to 1 pint per acre. Spray when grain is in full tiller stage (usually 4 to 8 inches tall) but before the boot stage and boot to dough stage. For improved control of difficult weeds including wild garlic and wild onion or under dry or cool conditions, apply up to 2 pints per acre. Wild garlic and wild onion may not be killed but dockage should be reduced. Do not use higher rates unless possible crop injury will be acceptable. Consult State Agricultural Experiment Station or Extension Service Weed Specialists for recommendations or suggestions to fit local conditions.

Spring Seeded Oats: Apply ½ pint per acre at the full tiller stage but before the early boot stage. Oats are less tolerant to 2,4-D than wheat or barley and are more likely to suffer some injury.

Fall Seeded Oats (Southern) Grown for Grain: Apply ¾ to 1¼ pints per acre after full tillering but before the early boot stage. Some difficult weeds may require higher rates for maximum control but crop injury may result. Do not spray during or immediately following cold weather.

Preharvest Treatment: Apply 1 to 2 pints per acre when grains are in the hard dough stage to control large weeds that may interfere with harvest. Best results will be obtained when soil moisture is sufficient to cause succulent weed growth. **NOTE:** Do not feed treated straw to livestock.

WEED CONTROL IN CORN: Use one of the following three programs. **Preemergence:** Apply 1 to 2 quarts per acre to soil anytime after planting but before corn emerges. Only emerged broadleaved weeds are likely to be controlled. Do not apply more than 1 quart per acre unless the increased risk of crop injury can be tolerated. Do not use on light sandy soil. **Emergence:** Apply 1 pint per acre just as corn plants are breaking ground. **Postemergence:** After emergence of corn use ½ pint per acre. Application of ¾ to 1 pint per acre may be needed for maximum control of some weeds but such rates are more likely to injure the corn. If corn is over 8 inches tall, use drop nozzles to keep the spray off the corn foliage as much as possible. Do not apply from the tasseling to dough stage. Do not use with oil, atrazine or other adjuvants. Crop injury is more likely to occur if corn is growing rapidly under high temperature and high soil moisture conditions. To reduce breakage of stalks from temporary brittleness caused by 2,4-D delay cultivation for 8 to 10 days after treatment. Do not forage or feed corn fodder for 7 days following application. NOTE: Hybrids vary in response to 2,4-D and some are easily injured. Safety only varieties known to be tolerant to 2,4-D. Contact seed company or your Agricultural Experiment Station or Extension Service weed specialists for this information.

PREHARVEST CORN TREATMENT: After the hard dough or denting sta
per acre by air or ground equipment to suppress perennial weeds. c
production, and control tall-weeds such as bindweed, cocklebur, do
ragweed, sunflower, velvetleaf and vines that interfere with harvesting. D
corn fodder for 7 days following application.

CONTROL OF WILD GARLIC AND WILD ONION IN STUBBLE GRAIN FI
harvest of small grain, soybeans, corn or grain sorghum, wild garlic a
produce new fall growth. This should be sprayed with 2 to 3 quarts per
Concentrate herbicide. This is a useful practice as one part of a control pr
for 7 days following application.

WEED CONTROL IN SORGHUM (MILO): Apply ½ pint per acre when sorg
tall. A higher rate of ¾ to 1 pint per acre may be needed to control some w
for crop injury is likewise increased. Do not use with oil. Do not treat bet
inches tall nor during the boot, flowering or early dough stages. If sorg
inches, use drop nozzles to keep the spray off the foliage as much as poss
injury may occur under conditions of high soil moisture and high air ter
vary in tolerance to 2,4-D and some hybrids are quite sensitive. Spray only
tolerant to 2,4-D. Contact seed company or your Agricultural Experiment
Service weed specialists for this information.

GRASS SEED CROPS: Use 1 to 1½ pints per acre in the amount of water
application by air or ground equipment. Apply to established stands in sp
early boot stage. Do not spray in boot stage. New spring seedings may be t
rate after the grasses have at least five leaves. Perennial weed regrowth r
fall.

WEED AND BRUSH CONTROL IN RANGELAND AND GRASS PASTURES
dairy animals on treated areas within 7 days after application. Do not use c
clover, or other legumes. Do not use on newly seeded areas until grass is
not use from early boot to milk stage where grass seed production is de

Bitterweed, Broomweed, Croton, Docks, Kochia, Marshelder, Muskthis
leaf Weeds: Use 2 quarts of ESTERON 99 Concentrate per acre in the amo
for uniform application. If the weeds are young and growing actively,
provide control of some species. Deeprooted perennial weeds may rec
ments in the same year or in subsequent years.

Wild Garlic and Wild Onion. Apply 2 to 3 quarts per acre, making three app
fall or spring-fall-spring) starting in late fall or early spring.

Weed Control in Newly Sprigged Coastal Bermudagrass: Apply 1 t
preemergence and or postemergence.

Sand Shinnery Oak and Sand Sagebrush: On the oak, use 1 quart in 5
gallons of water plus 1 gallon of oil per acre. Apply by aircraft between Ma
the sagebrush, use 1 quart in 3 gallons of oil per acre and apply by aircraft
expanded and the brush is actively growing.

Big Sagebrush and Rabbitbrush: Use 2 to 3 quarts per acre in 2 to 3 gall
gallons of oil-water emulsion spray. For rabbitbrush, the 3 quart rate is usu
should be leafed out and growing actively when treated. Retreatment ma

Chamise, Manzanita, Buckbrush, Coastal Sage, Coyotebrush and Cert
Species: Use 2 to 3 quarts per acre in 5 to 10 gallons of water. One gallo
included in the spray mixture for added effectiveness. Make applications
equipment to obtain uniform spray coverage. For effective control, the
leafed out and growing actively when sprayed. Retreatment may be need

WOODY PLANT CONTROL IN NON-CROP AREAS: To control species su
rights-of-way, fencerows, roadsides, and along drainage ditch banks, spr
feet tall after spring foliage is well developed, using 3 to 4 quarts of ESTE
in 100 gallons of water and wetting all parts of the brush including foliage.
may require up to 400 gallons of spray per acre for adequate coverage of
Make application in such a way as to prevent drift of the spray off the
Spraying can be effective at any time up to 3 weeks before frost as long as
sufficient for active growth of the brush. Control will be less effective in mi
dry weather when soil moisture is deficient and plants are not actively gro
agent may be added to the spray, if needed for increased effectiveness.

Forest Site Preparation: For control of susceptible broadleaf weeds and
planted in forests, use 1.5 to 8 quarts per acre of ESTERON 99 Conc
sufficient spray volume for good plant coverage, usually 6 to 25 gallons
made by air or ground (hand gun, boom, or powered knapsack sprayer). T
diesel oil per acre or a suitable surfactant or penetrant may be added to im

Forest Conifer Release: For applications in late winter or spring to
deciduous brush species, such as alder, willow, poplars, cascara, cherry
vine maple during early growth and before conifer budbreak, use ESTERC
rates up to 3 quarts per acre in diesel or stove oil by air or ground in sufficie
good plant coverage, usually 6 to 25 gallons. **Do not use in plantations wh**
among the desired species.

For treatment before conifer budbreak to control susceptible evergreen br
tanoak, madrone, chinquapin, ceanothus spp. and manzanita or dec
leafout or broadleaf weeds, use ESTERON 99 Concentrate at rates up to 3 c
or with 0.5 to 2.0 gallons per acre of diesel or similar oil or suggeste
surfactants or penetrants. After conifer budbreak, ESTERON 99 Conc
surfactant or penetrant can be used at rates up to 2 quarts per acre but
suppression of the conifer growth. Use sufficient volume of spray for gooc
usually 6 to 25 gallons. Some species of pine may be seriously injured b
growth stages.

After conifer species such as white pine, ponderosa pine, jack pine, red
white spruce, red spruce, and balsam fir cease growth and harden off and
growing in late summer, 1.5 to 3.0 quarts of ESTERON 99 Concentrate per a
to obtain good plant coverage may be applied by air or ground to contro
hardwood species such as alder, aspen, birch, haze, and willow. Since
cause occasional conifer injury, do not use if such injury cannot be tolera

Directed Sprays in Conifer Plantations (including pine): Apply ESTER
herbicide at any time brush or broadleaf weeds are susceptible by directi
conifers to avoid contact of needles with unnecessary amounts of spray. Ra
Concentrate are not to exceed 4 quarts per acre in oil, oil-water, or wate
gallons per acre.

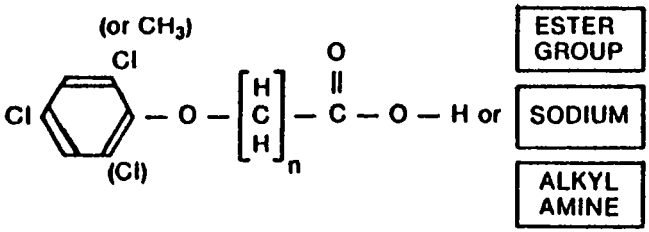
WEED CONTROL IN NON-CROP AREAS SUCH AS LAWNS, GOLF COURS
PARKS, AIRFIELDS, ROADSIDES, VACANT LOTS, DRAINAGE DITCH B
quarts of ESTERON 99 Concentrate per acre in the amount of water
application. Usually 2 quarts per acre provides good weed control under
Treat when weeds are young and growing well. Do not use on golf greens
other broadleaf herbaceous ground covers. Do not use on creeping grass
St. Augustine except for spot treating noxious weed. Seed-down until grass
Reseeding of treated areas should be delayed following treatment. With
reseeded in the fall, with fall application reseed in the spring. Legumes are
tolerant. Do not treat areas where legumes are desired. Deeprooted pe
may require repeated treatments in the same season or in subsequent years.

TULE (BULRUSH) AND OTHER RUSHES Mix 2 quarts of ESTERON 99 Co
and 3 gallons of diesel oil or kerosene, then add this mixture to 100 gallons o

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CHLOROPHENOXY COMPOUNDS

GENERAL CHEMICAL STRUCTURE



COMMON COMMERCIAL PESTICIDE PRODUCTS

Several hundred commercial products contain chlorophenoxy herbicides in various concentrations and combinations. Following are names of widely advertised formulations. In some cases, the same name is used for products with different ingredients. Exact composition must therefore be determined from product label.

2,4-D, or 2,4-dichlorophenoxyacetic acid (Weedone†, Agrotec, Amoxone, Aqua-Kleen, BH 2,4-D, Chipco Turf Herbicide "D", Chloroxone, Crop Rider, D50, Dacamine 4D, Ded-Weed, Desormone, Dinoxol, DMA4, Dormone, Emulsamine BK, Emulsamine E-3, Envert DT or 171, Esteron 99 Concentrate, Esteron Four, Esteron Brush Killer, Estone, Fernoxone, Fernimine, Ferxone, Fernesta, Formula 40, Hedonal, Herbidal, Lawn-Keep, Macondray, Miracle, Netagrone 600, Pennamine D, Planotox, Plantgard, Rhodia, Salvo‡, Spritz-Hormin/2,4-D, Spritz-Hormit/2,4-D, Superormone Concentre, Super D Weedone, Transamine, U46, Verton 2D, Visko-Rhap, Weed-B-Gon, Weedar, Weed-Rhap, Weed Tox, Weedtrol, De broussaillant 600, Lithate, Dicotox, Field Clean Weed Killer). 2,4-DB is the butyric acid homologue of 2,4-D. Dichlorprop is the propionic acid homologue.

2,4,5-T or 2,4,5-trichlorophenoxyacetic acid (Brush-Rhap, Dacamine 4T, Debroussaillant Concentre, Ded-Weed Brush Killer, Esteron 245, Fence Rider, Forron, Inverton 245, Line Rider, Spontox, Super D Weedone, Tormona, Transamine, Trinoxol, Trioxone, U46, Veon 245, Verton 2T, Weedar, Weedone Envert T).

Common mixtures of 2,4-D and 2,4,5-T are: Dacamine 2D/2T, Esteron Brush Killer, Rhodia Low Volatile Brush Killer No. 2, U46 Special, Tributon, Visko-Rhap LV2D-2T, and Transamine.

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† A product of identical name containing pentachlorophenol (Chapter 4) as the active ingredient has been discontinued by Amchem Products Co.

‡ A product of identical name marketed by the Crystal Chemical Company contains cacodylic acid as the active ingredient (Chapter 10).

2,4,5-TP (Silvex) is the propionic acid homologue of 2,4,5-T. Kuron is a low volatile ester of 2,4,5-TP. 2,4,5-TB is the butyric acid homologue of 2,4,5-T. Fenac or chlorfenac is 2,3,6-trichlorophenylacetic acid. Dicamba (Banvel) is dichloroanisic acid. MCPA, MCPB, MCPB-Ethyl, MCPCA and MCPP (Mecoprop) are 2-methyl, 4-chlorophenoxy aliphatic acids and esters.

TOXICOLOGY

Some of the chlorophenoxy acids, salts, and esters are moderately irritating to skin, eyes, and respiratory and gastrointestinal linings. In a few individuals, local depigmentation has apparently resulted from prolonged and repeated dermal contact with chlorophenoxy materials.

The chlorophenoxy compounds are absorbed across the gut wall, lung, and skin. They are not significantly fat storable. Excretion occurs within hours, or at most, days, primarily in the urine.

Given in large doses to experimental animals, 2,4-D causes vomiting, diarrhea, anorexia, weight loss, ulcers of the mouth and pharynx, and toxic injury to the liver, kidneys, and central nervous system. Myotonia (stiffness and incoordination of hind extremities) develops in some species and is apparently due to CNS damage: demyelination has been observed in the dorsal columns of the cord, and EEG changes have indicated functional disturbances in the brains of heavily dosed experimental animals.

Ingestion of large amounts of chlorophenoxy acids has resulted in severe metabolic acidosis in humans. Such cases have been associated with electrocardiographic changes, myotonia, muscle weakness, myoglobinuria, and elevated serum creatine phosphokinase, all reflecting injury to striated muscle. Because chlorophenoxy acids are weak uncouplers of oxidative phosphorylation, extraordinary doses may produce hyperthermia from increased production of body heat.

PolyChlorinated DibenzoDioxin (CDD) compounds are generated in the synthesis of 2,4,5-T. The 2,3,7,8-Tetra CDD form is extraordinarily toxic to multiple mammalian tissues. Hexa- hepta-, and octa-compounds exhibit less systemic toxicity, but are the likely cause of chloracne (a chronic, disfiguring skin condition) seen in workers engaged in the manufacture of 2,4,5-T, and certain other chlorinated organic compounds. Although toxic effects, notably chloracne, have been observed in manufacturing plant workers, they have not been observed in formulators or applicators regularly exposed to 2,4,5-T.

The medical literature contains several reports of peripheral neuropathy following what seemed to be minor dermal exposures to 2,4-D. It is not certain that exposures to other neurotoxicants were entirely excluded in these cases. Single doses of 5 mg/kg body weight of 2,4-D and 2,4,5-T have been administered to human subjects without any adverse effects. One subject consumed 500 mg of 2,4-D per day for 3 weeks without experiencing symptoms or signs of illness.

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FREQU[illegible] SYMPTOMS AND SIGNS OF POISONING

Chlorophenoxy compounds are moderately **IRRITATING** to skin and mucous membranes. Inhalation of sprays may cause burning sensations in the nasopharynx and chest, and coughing may result. Prolonged inhalation sometimes causes dizziness.

When **INGESTED**, high concentrations of chlorophenoxy compounds may irritate the mouth, throat, and gastrointestinal tract. Prompt **EMESIS, CHEST PAIN** (from esophagitis), **ABDOMINAL PAIN**, and **DIARRHEA** commonly ensue. Injury to the GI tract does not usually progress to ulceration or perforation. Absorbed chlorophenoxy compounds have caused **FIBRILLARY MUSCLE TWITCHING**, skeletal muscle tenderness, and **MYOTONIA** (stiffness of muscles of the extremities). Ingestion of very large amounts has produced **METABOLIC ACIDOSIS**, fever, tachycardia, hyperventilation, vasodilatation and sweating. Particular cases have been characterized by coma and convulsions.

CONFIRMATION OF DIAGNOSIS

Gas-liquid chromatographic methods are available for detecting and measuring the chlorophenoxy compounds in blood and urine. These analyses are useful in confirming and assessing the magnitude of chlorophenoxy absorption. Urine samples should be collected as soon as possible after exposure because the herbicides may be almost completely excreted in 24-72 hours, depending on the extent of toxicant absorption. Analyses can be performed at special laboratories operated by state health departments, chemical companies, universities, and government facilities. If circumstances indicate strongly that excessive exposure to any of these compounds has occurred, initiate appropriate treatment measures immediately, not waiting for chemical confirmation of toxicant absorption.

TREATMENT

1. **BATHE** and **SHAMPOO** with soap and water to remove chemicals from skin and hair. Individuals with chronic skin disease or known sensitivity to chemicals should either avoid using these herbicides or take extraordinary measures to avoid contact.
2. **FLUSH** contaminating chemicals from eyes with copious amounts of clean water for 10-15 minutes.
3. If symptoms of illness occur during or following inhalation of spray, **REMOVE** victim **FROM CONTACT** with the material for at least two days. Allow subsequent contact with chlorophenoxy compounds only if effective respiratory protection is practiced.
4. IF substantial amounts of chlorophenoxy compounds have been **INGESTED**, spontaneous emesis usually occurs. Ordinarily, this empties the stomach as effectively as intubation and lavage. If vigorous emesis

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has not occurred and **IF VICTIM IS FULLY ALERT,** induce **EMESIS** with **SYRUP OF IPECAC** (adults 12 years and older, 30 ml; children under 12 years, 15 ml), followed by 1-2 glasses of water. Following emesis, administer 30-50 gm of **ACTIVATED CHARCOAL** in a slurry of 6-8 ounces tap water, to limit absorption of herbicide remaining in the gut.

5. **IF CONSCIOUSNESS LEVEL IS DEPRESSED** or other signs of **NEUROTOXICITY** appear, **SUSPECT** additional or alternative ingested toxicants. Evacuate the stomach by **INTUBATION, ASPIRATION,** and **LAVAGE.** Because petroleum distillates are commonly included in chlorophenoxy formulations, gastric intubation incurs a risk of hydrocarbon pneumonitis from aspiration. For this reason:

   A. If victim is unconscious or obtunded and facilities are at hand, insert an **ENDOTRACHEAL TUBE** (cuffed, if available) prior to gastric intubation.
   B. Keep victim's **HEAD BELOW LEVEL OF THE STOMACH** during intubation and lavage (Trendelenburg, or left lateral decubitus, with head of table tipped downward). Keep victim's head turned to left.
   C. **ASPIRATE PHARYNX** as regularly as possible to remove gagged or vomited stomach contents.

   After aspiration of gastric contents and washing of stomach, instill 30-50 gm of **ACTIVATED CHARCOAL** in 3-4 ounces of water through the stomach tube to limit absorption of remaining toxicant. Do **NOT** instill milk, cream, or other materials containing vegetable or animal fats, as these are likely to enhance absorption.

6. If bowel movement has not occurred in 4 hours and patient is fully conscious, give **SODIUM SULFATE,** 0.25 gm/kg, as a cathartic. Magnesium sulfate and citrate, in comparable dosages, are equally suitable if renal function is adequate. Retained magnesium may depress CNS function.

7. In **SEVERE POISONINGS** by very large amounts of ingested chlorophenoxy acids, forced **ALKALINE DIURESIS** may save the victim's life. Assess serum electrolyte concentrations, and serum and urine pH. If a metabolic acidosis is present, infuse solutions of sodium bicarbonate at rates sufficient to keep the urine distinctly alkaline, continuing until plasma concentrations of chlorophenoxy compounds are less than about 10 μg/ml. [Prescott, L F. et al., *Br. J. Clin. Pharmacol.* 7:11 (1979)]

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PESTICIDE & TOXIC CHEMICAL NEWS

Weekly reports on hazardous wastes, pesticides, toxic substances and general issues of regulation and legislation

EPA NOTES INTENT TO END ALL USES OF 2,4,5-T AND SILVEX

Following Dow Chemical Company's withdrawal from the 2,4,5-T and silvex cancellation hearing and voluntary cancellation of registration of the products, EPA has issued notices to other registrants seeking to end all uses of the pesticides without more hearings (See Aug. 10, Page 5; and Aug. 31, Page 2). To continue registrations, hearings have to be requested within 30 days of the agency's Oct. 14 notice.

The Oct. 14 intent to cancel notice covers all remaining non-suspended registrations of the products, those labeled for rice, range, sugarcane, orchard and miscellaneous non-crop uses, manufacturing use, and technical registrations.

At the same time, EPA issued an enforcement policy statement "to prohibit the sale, distribution, importation, or other transfer of unregistered 2,4,5-T and silvex products, except as specifically allowed by FIFRA Section 3(b)."

The agency said it was also "requiring each registrant of a 2,4,5-T or silvex product to amend the confidential statement of formula for that product to identify the source of each active ingredient in the product."

EPA said failure to request a hearing "will not prevent the distribution or sale of existing stocks of certain products whose registrations are cancelled. The agency will permit the continued distribution or sale of existing stocks of 2,4,5-T and silvex products which are labeled for end uses subject to this notice, and whose registrations are cancelled pursuant to this notice, for no more than one year from the effective date of such cancellation. However, this provision will apply only to those existing stocks of 2,4,5-T and silvex products which were packaged and labeled for such end uses and released for shipment prior to the date of receipt of this notice by the registrant or publication of this notice in the Federal Register, whichever occurs first. The agency will not permit the continued distribution, sale, or use of any cancelled 2,4,5-T or silvex product labeled for any end use subject to the 1979 emergency suspension order or for manufacturing use." The agency's letter to registrants continued:

> "EPA notes that many registrants receiving this letter may hold registrations for products which were originally labeled for suspended uses of 2,4,5-T or silvex. Registrants of products which were labeled for one or more suspended uses and who elect not to participate in any further hearing concerning such products may meet the qualifications for receiving indemnification payment under FIFRA Section 15(a)."

The agency also noted provision for EPA disposal of the cancelled pesticides. The letter to the registrants signed by Edwin L. Johnson, OPP Director, stated:

> "The time-consuming and costly nature of continued litigation supports my belief that it will be in the public interest if the 2,4,5-T/silvex cancellation proceeding can be concluded without the taking of additional testimony.

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"If the hearing resumes, it is estimated that it will take at least one year to bring the administrative proceedig to conclusion, allowing for supplemental pre-trial activities including discovery, presentation of additional testimony, briefing, and issuance of recommended and final decisions, plus another year or more to resolve judicial appeals.

"While much of the evidence of risks associated with use of 2,4,5-T and silvex has been presented, EPA anticipates that additional risk testimony would have to be presented concerning information which has accumulated during the two and one-half year period since the hearing was suspended.

"In addition, since the benefits presentation had barely gotten underway when the hearing was recessed, vitually the entire benefits case will have to be presented."

The agency's Section 6(b)(1) cancellation notice said, "If a registrant whose 2,4,5-T or silvex product is labeled for more than one of the uses subject to this notice or other adversely affected person elects to request a hearing concerning some but not all affected uses of the product, the registrant also must submit to the Registration Division, OPP, an application to amend the registration of such product to delete all other affected uses within the same 30-day period within which a hearing may be requested. Failure to submit such an application for amended registration will result in cancellation of the entire registration of the product by operation of law." It continued:

"If a registrant or other adversely affected person requests a hearing pursuant to this notice for y 2,4,5-T or silvex product for which no hearing has previously been requested and the labeling submitted for that product has not been previously amended to delete all uses which were subject to the emergency suspension and Section 6(b)(1) cancellation notices for 2,4,5-T and silvex issued in 1979, the registrant also must submit to the Registration Division of the Office of Pesticide Programs an application to amend such labeling to delete such previously cancelled uses within the same 30-day period within which a hearing may be requested. Failure to submit such an application for amended registration will result in cancellation of the entire registration of the pesticide.

"Consequences of failure to file in a timely and effective manner. If no hearing has been requested within the applicable 30-day period for any 2,4,5-T or silvex product which is labeled for one or more uses subject to this notice and the registrant has not submitted to EPA an application to amend the registration of such product to delete all uses subject to this notice, the entire registration of the product will be cancelled. However, if a registrant does not request a hearing for a 2,4,5-T or silvex product which is labeled for one or more uses subject to this notice, but did previously request a hearing concerning cancellation of one or more uses of that product which were subject to the emergency suspension notices and notice of intent to cancel 2,4,5-T and silvex issued in 1979, the registrant has the right to continue to participate in a hearing with respect to the latter uses. At the conclusion of the 2,4,5-T/silvex cancellation proceeding, reinstatement of such uses will be granted to the extent permitted by the final adjudicatory order. Any registrant or former registrant who requests reinstatement of a registration for such uses pursuant to such a final adjudicatory order will not be required to comply with Section 3(c)(1)(D) of FIFRA in order for such reinstatement to be granted."

Dow, in ending its participation in the hearing and withdrawing its hearing request, said, "2,4,5-T and silvex are safe, effective herbicides that neither pose nor threaten unreasonable adverse effects on the environment and are therefore properly registered under the FIFRA. Scientific panels, regulatory authorities and judicial decision makers in this country and throughout the world, including EPA's own Scientific Advisory Panel in reviewing parts of this proceeding, have reached similar conclusions."

The company further stated, "in taking this action, Dow is hopeful that resources heretofore devoted to this proceeding will be freed for more constructive use in addressing the broader issues of the presence of chemicals, including dioxin, in the environment. Further pursuit of this proceeding could detract from that broader inquiry."

Johnson, responding to Dow's request for voluntary cancellations, accepted the request, effective immediately. He said, "existing stocks of such products which were formulated, packaged, labeled only for end uses which have not previously been suspended, and released for shipment by your firm on or before Oct. 14, 1983, may be distributed and sold until Oct. 14, 1984, but not thereafter."

The agency official concluded, "while we do not agree with all of the statements concerning 2,4,5-T and silvex . . ., we do agree that it is in the public interest to bring the 2,4,5-T/silvex proceeding to a close."

In an Oct. 14 letter to Alvin L. Alm, EPA Deputy Administrator, Dow's K.R. McKennon, Group Vice President, said, "fortunately, progress made by Dow and others in herbicide product development since 1979 has also resulted in substitute products being available for most uses of 2,4,5-T and silvex. Dow is convinced that these substitutes are now sufficiently available to satisfy its obligation to customers." He continued:

> "With the issue of 2,4,5-T and silvex behind us, Dow hopes that it can help to forge a lasting partnership with EPA and the many scientific groups, environmental organizations, and industrial companies having an interest and expertise in dioxin matters."

The cancellation notice and enforcement policy were published in the Oct. 18 Federal Register.

Occupational Health Guideline for 2,4,5-T *

INTRODUCTION

This guideline is intended as a source of information for employees, employers, physicians, industrial hygienists, and other occupational health professionals who may have a need for such information. It does not attempt to present all data; rather, it presents pertinent information and data in summary form.

SUBSTANCE IDENTIFICATION

- Formula: $Cl_3C_6H_2OCH_2COOH$
- Synonyms: 2,4,5-Trichlorophenoxyacetic acid
- Appearance and odor: Colorless to tan odorless solid.

PERMISSIBLE EXPOSURE LIMIT (PEL)

The current OSHA standard for 2,4,5-T is 10 milligrams of 2,4,5-T per cubic meter of air (mg/m^3) averaged over an eight-hour work shift.

HEALTH HAZARD INFORMATION

- **Routes of exposure**

2,4,5-T can affect the body if it is inhaled or if it comes in contact with the eyes or skin. It can also affect the body if it is swallowed.

- **Effects of overexposure**

Exposure to 2,4,5-T may cause abdominal pain, nausea, vomiting, diarrhea, and blood in the stool. It may also cause irritation of the skin. Common contaminants of commercial preparations of 2,4,5-T may cause acne and liver damage. Animal experiments have shown that these contaminants may produce damage in unborn rats.

- **Reporting signs and symptoms:**

A physician should be contacted if anyone develops any signs or symptoms and suspects that they are caused by exposure to 2,4,5-T.

- **Recommended medical surveillance**

The following medical procedures should be made available to each employee who is exposed to 2,4,5-T at potentially hazardous levels:

1. Initial Medical Examination:

—A complete history and physical examination: The purpose is to detect pre-existing conditions that might place the exposed employee at increased risk, and to establish a baseline for future health monitoring. Examination of the liver and attention to gastrointestinal complaints should be stressed. The skin should be examined for evidence of chronic disorders.

2. Periodic Medical Examination: The aforementioned medical examinations should be repeated on an annual basis.

- **Summary of toxicology**

2,4,5-T (2,4,5-trichlorophenoxyacetic acid) is of low toxicity. The oral LD50 for dogs is in the range of 100 mg/kg or higher; effects are limited to a slight or moderate stiffness in the hind legs with development of ataxia. Contaminants of commercial preparations of 2,4,5-T have been 2,3,7,8-tetrachlorodibenzo-p-dioxin, a potent animal teratogen, and 2,3,6,7-tetrachlorodibenzo-p-dioxin (TCDD), a potent acnegenic agent which is hepatotoxic in animals; they are present as unwanted side products of synthesis of 2,4,5-T. In a study of 73 workers in a 2,4,5-T manufacturing plant, 13 had moderate to severe acneform dermatitis (chloracne) and 22 had gastrointestinal complaints such as nausea, vomiting, diarrhea, abdominal pain, or blood in the stool; no significant liver dysfunction was found; although no air sample results were reported, the chloracne was thought to be a result of exposure to TCDD. 2,4,5-T dust is a slight irritant of the skin.

CHEMICAL AND PHYSICAL PROPERTIES

- **Physical data**

1. Molecular weight: 255.5
2. Boiling point (760 mm Hg): Decomposes above melting point
3. Specific gravity (water = 1): Greater than 1
4. Vapor density (air = 1 at boiling point of 2,4,5-T): Not applicable
5. Melting point: 158 C (316 F) (decomposition)

These recommendations reflect good industrial hygiene and medical surveillance practices and their implementation will assist in achieving an effective occupational health program. However, they may not be sufficient to achieve compliance with all requirements of OSHA regulations.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service Centers for Disease Control
National Institute for Occupational Safety and Health

U.S. DEPARTMENT OF LABOR
Occupational Safety and Health Administration

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6. Vapor pressure at 20 C (68 F): Essentially zero

7. Solubility in water, g/100 g water at 20 C (68 F): 0.03

8. Evaporation rate (butyl acetate = 1): Not applicable

• **Reactivity**

1. Conditions contributing to instability: Temperatures above 158 C (316 F) may cause sealed metal containers to burst.

2. Incompatibilities: None.

3. Hazardous decomposition products: Toxic gases and vapors (such as hydrogen chloride and carbon monoxide) may be released when 2,4,5-T decomposes.

4. Special precautions: None.

• **Flammability**

1. Not combustible

• **Warning properties**

2,4,5-T is not known to be an eye irritant.

MONITORING AND MEASUREMENT PROCEDURES

• **General**

Measurements to determine employee exposure are best taken so that the average eight-hour exposure is based on a single eight-hour sample or on two four-hour samples. Several short-time interval samples (up to 30 minutes) may also be used to determine the average exposure level. Air samples should be taken in the employee's breathing zone (air that would most nearly represent that inhaled by the employee).

• **Method**

An analytical method for 2.4,5-T is in the *NIOSH Manual of Analytical Methods.* 2nd Ed., Vol. 5, 1979, available from the Government Printing Office, Washington, D.C. 20402 (GPO No. 017-033-00349-1).

RESPIRATORS

• Good industrial hygiene practices recommend that engineering controls be used to reduce environmental concentrations to the permissible exposure level. However, there are some exceptions where respirators may be used to control exposure. Respirators may be used when engineering and work practice controls are not technically feasible, when such controls are in the process of being installed, or when they fail and need to be supplemented. Respirators may also be used for operations which require entry into tanks or closed vessels, and in emergency situations. If the use of respirators is necessary, the only respirators permitted are those that have been approved by the Mine Safety and Health Administration (formerly Mining Enforcement and Safety Administration) or by the National Institute for Occupational Safety and Health.

• In addition to respirator selection, a complete respiratory protection program should be instituted which includes regular training, maintenance, inspection, cleaning, and evaluation.

SANITATION

• Eating and smoking should not be permitted in areas where 2,4,5-T is handled, processed, or stored.

• Employees who handle 2,4,5-T should wash their hands thoroughly with soap or mild detergent and water before eating, smoking, or using toilet facilities.

COMMON OPERATIONS AND CONTROLS

The following list includes some common operations in which exposure to 2,4,5-T may occur and control methods which may be effective in each case:

Operation	Controls
Formulation of herbicides and plant hormones	Process enclosure; local exhaust ventilation; personal protective equipment
Application as herbicide, defoliant, and plant hormone	Personal protective equipment
Manufacture of 2,4,5-T	Process enclosure; local exhaust ventilation; personal protective equipment

EMERGENCY FIRST AID PROCEDURES

In the event of an emergency, institute first aid procedures and send for first aid or medical assistance.

• **Eye Exposure**

If 2,4,5-T gets into the eyes, wash eyes immediately with large amounts of water, lifting the lower and upper lids occasionally. If irritation is present after washing, get medical attention. Contact lenses should not be worn when working with this chemical.

• **Skin Exposure**

If 2,4,5-T or liquids containing 2,4,5-T get on the skin, wash the contaminated skin using soap or mild detergent and water. If 2,4,5-T or liquids containing 2,4,5-T soak through the clothing, remove the clothing and wash the skin using soap or mild detergent and water. If irritation is present after washing, get medical attention.

• **Breathing**

If a person breathes in large amounts of 2,4,5-T, move the exposed person to fresh air at once. If breathing has stopped, perform artificial respiration. Keep the affected person warm and at rest. Get medical attention as soon as possible.

• **Swallowing**

When 2,4,5-T or liquids containing 2,4,5-T have been swallowed and the person is conscious, give the person large quantities of water immediately. After the water has been swallowed, try to get the person to vomit by having him touch the back of his throat with his finger. Do not make an unconscious person vomit. Get medical

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attention immediately.

• Rescue

Move the affected person from the hazardous exposure. If the exposed person has been overcome, notify someone else and put into effect the established emergency rescue procedures. Do not become a casualty. Understand the facility's emergency rescue procedures and know the locations of rescue equipment before the need arises.

SPILL AND DISPOSAL PROCEDURES

• Persons not wearing protective equipment and clothing should be restricted from areas of spills until cleanup has been completed.

• If 2,4,5-T is spilled, the following steps should be taken:

1. Ventilate area of spill.
2. Collect spilled material in the most convenient and safe manner and deposit in sealed containers for reclamation, or for disposal in a secured sanitary landfill. Liquid containing 2,4,5-T should be absorbed in vermiculite, dry sand, earth, or a similar material.

• Waste disposal method:

2,4,5-T may be disposed of in sealed containers in a secured sanitary landfill.

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* SPECIAL NOTE

The International Agency for Research on Cancer (IARC) has evaluated the data on this chemical and has concluded that it causes cancer. See *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Volume 15, 1977.

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Toxicological Profile for

2,3,7,8-TETRACHLORO-DIBENZO-*p*-DIOXIN

Draft

For Public Comment

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Agency for Toxic Substances and Disease Registry
U.S. Public Health Service

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Comment Period Ends:

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DRAFT

TOXICOLOGICAL PROFILE FOR 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

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DISCLAIMER

Mention of company name or product does not constitute endorsement by the Agency for Toxic Substances and Disease Registry.

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FOREWORD

The Superfund Amendments and Reauthorization Act of 1986 (Public Law 99-499) extended and amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law (also known as SARA) directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare toxicological profiles for hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List and which pose the most significant potential threat to human health, as determined by ATSDR and the Environmental Protection Agency (EPA). The list of the 100 most significant hazardous substances was published in the *Federal Register* on April 17, 1987.

Section 110 (3) of SARA directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. Each profile must include the following content:

> "(A) An examination, summary, and interpretation of available toxicological information and epidemiologic evaluations on the hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects,
>
> (B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure which present a significant risk to human health of acute, subacute, and chronic health effects, and
>
> (C) Where appropriate, an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans."

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and EPA. The guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary, but no less often than every three years, as required by SARA.

The ATSDR toxicological profile is intended to characterize succinctly the toxicological and health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature that describes a hazardous substance's toxicological properties. Other literature is presented but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

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Each toxicological profile begins with a public health statement, which describes in nontechnical language a substance's relevant toxicological properties. Following the statement is material that presents levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Research gaps in toxicologic and health effects information are described in the profile. Research gaps that are of significance to protection of public health will be identified by ATSDR, the National Toxicology Program of the Public Health Service, and EPA. The focus of the profiles is on health and toxicological information; therefore, we have included this information in the front of the document.

The principal audiences for the toxicological profiles are health professionals at the federal, state, and local levels, interested private sector organizations and groups, and members of the public. We plan to revise these documents in response to public comments and as additional data become available; therefore, we encourage comment that will make the toxicological profile series of the greatest use.

This profile reflects our assessment of all relevant toxicological testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, EPA, the Centers for Disease Control, and the National Toxicology Program. It has also been reviewed by a panel of nongovernment peer reviewers and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

James O. Mason

James O. Mason, M.D., Dr. P.H.
Assistant Surgeon General
Administrator, ATSDR

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1. PUBLIC HEALTH STATEMENT

1.1 WHAT IS DIOXIN?

The chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is commonly called dioxin. Dioxin, however, is an inaccurate colloquial name for 2,3,7,8-TCDD. It is a colorless solid with no distinguishable odor. 2,3,7,8-TCDD is neither known to occur naturally nor is it intentionally manufactured by any industry, except as a reference standard. It can be inadvertently produced in very small amounts as an impurity during the manufacture of certain herbicides and germicides, and during the incineration of municipal and industrial wastes. At the present time, 2,3,7,8-TCDD is not used for any purpose other than scientific research.

1.2 HOW MIGHT I BE EXPOSED TO 2,3,7,8-TCDD?

The main environmental sources of 2,3,7,8-TCDD are:

- Production and use of herbicides containing 2,4,5-trichlorophenoxy acids (2,4,5-T)
- Production and use of 2,4,5-trichlorophenol
- Production and use of hexachlorophene as a germicide
- Incineration of municipal and certain industrial wastes
- Small amounts formed during the burning of wood in the presence of chlorine
- Accidental transformer/capacitor fires involving chlorinated benzenes and biphenyls
- Exhaust from automobiles powered with leaded gasoline
- Improper disposal of certain chlorinated chemical wastes

Information on the level of 2,3,7,8-TCDD in ambient air is not available. Detection of 2,3,7,8-TCDD in drinking water has not been reported. The concentrations of 2,3,7,8-TCDD in most rural soils is undetectable, but it can be present at trace levels in urban soils. In a waste oil-contaminated soil in Missouri, the 2,3,7,8-TCDD level was more than one million times higher than in soils from normal urban areas. 2,3,7,8-TCDD was detected in fish obtained from the contaminated sections of Lake Ontario, Saginaw Bay, and the Michigan rivers. In human milk, 2,3,7,8-TCDD has not been detected in the United States; however, using more sensitive methods of measurement, it has been detected in several European countries. It has not been detected in any other foods.

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Consumer sources:

- Skin contact with surfaces such as soil or vegetation contaminated by the chemical
- Consumption of root vegetables grown in contaminated soils
- Inhalation of air near improperly maintained dump sites or municipal incinerators
- Consumption of fish, livestock, and cow's milk from contaminated areas
- Breast-fed babies nursed by mothers who have 2,3,7,8-TCDD in their breast milk
- Since both 2,4,5-T-containing herbicides and hexachlorophene have been withdrawn from consumer use, they no longer will be future sources of exposure; however, exposures to these compounds may occur from hazardous waste sites.

Occupational sources:

- Workers involved in the production or use of trichlorophenol and salts, hexachlorophene, and 2,4,5-T or other herbicides containing this chemical. The production of 2,4,5-T and 2,4,5-trichlorophenol, however, has been discontinued in the United States.
- Workers at certain municipal and industrial incinerators
- Workers at certain hazardous waste sites
- Workers involved in the cleanup of certain accidental capacitor/transformer fires and in the salvaging of transformers
- Workers involved in spraying phenoxy herbicides such as Agent Orange

1.3 HOW DOES 2,3,7,8-TCDD GET INTO MY BODY?

- Absorption through skin from contaminated soils and other materials
- Ingestion of 2,3,7,8-TCDD through the consumption of contaminated fish, cow's milk, and foodstuffs
- Breathing contaminated ambient air may contribute very small amounts to total body intake. Inhalation of particulates such as fly ash, however, may constitute a major source of exposure.
- Intake of 2,3,7,8-TCDD from the consumption of drinking water should be negligible.

1.4 HOW CAN 2,3,7,8-TCDD AFFECT MY HEALTH?

- In humans, 2,3,7,8-TCDD causes chloracne, a severe skin lesion that usually occurs on the head and upper body. Unlike common acne, chloracne is more disfiguring and often lasts for years after the initial exposure.

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- There is suggestive evidence that 2,3,7,8-TCDD causes liver damage in humans, as indicated by an increase in levels of certain enzymes in the blood. Animal studies have demonstrated severe liver damage in some species.
- There is suggestive evidence that 2,3,7,8-TCDD causes loss of appetite, weight loss, and digestive disorders in humans. Animal exposure to 2,3,7,8-TCDD results in severe loss of body weight just prior to death.
- Although never demonstrated in humans, in animal studies, 2,3,7,8-TCDD produced toxicity to the immune system. This toxicity can result in greater susceptibility to infection.
- Although never demonstrated in humans, in some animal species, exposure to 2,3,7,8-TCDD resulted in spontaneous abortions. The monkey is very sensitive to this toxic property of 2,3,7,8-TCDD.
- Although never demonstrated in humans, in rodents, exposure to 2,3,7,8-TCDD during pregnancy resulted in malformations in the offspring.
- 2,3,7,8-TCDD has been demonstrated to be a carcinogen in animals.

1.5 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 2,3,7,8-TCDD?

There is no common medical test available to demonstrate unequivocally that you have been exposed to 2,3,7,8-TCDD. It is believed that a blood test to detect certain enzymes indicating liver damage may be helpful in determining whether exposure has occurred. These tests do not indicate with certainty that you have been exposed to 2,3,7,8-TCDD, since other chemicals, as well as drinking alcohol, can cause similar results. When tests for these enzymes have been performed, changes in these enzymes were demonstrated only in some of the people suspected of 2,3,7,8-TCDD exposure.

There are other tests available, which are not commonly conducted by a physician but appear to more adequately indicate that you have been exposed to 2,3,7,8-TCDD. One test consists of removing a small piece of body fat by a simple surgical procedure; the fat is then analyzed for the presence of 2,3,7,8-TCDD. In another recently developed test, blood serum is obtained and analyzed for the presence of 2,3,7,8-TCDD. The initial study appears to indicate that the method is sensitive enough to detect background levels of 2,3,7,8-TCDD. If the levels of 2,3,7,8-TCDD are higher than the determined average for people in the United States, the test indicates that you have probably been exposed to more 2,3,7,8-TCDD than the average population. In addition, detection of 2,3,7,8-TCDD in mother's milk would also indicate exposure; the level of 2,3,7,8-TCDD in the milk may provide some indication of whether exposure was to background levels or if additional exposure occurred. This method has not been used widely to evaluate human exposure.

1.6 WHAT LEVELS OF EXPOSURE BY INGESTION AND BY SKIN CONTACT HAVE RESULTED IN HARMFUL HEALTH EFFECTS?

The graphs on the following pages show the relationship between exposure to 2,3,7,8-TCDD and known health effects. In the first set of graphs labeled "Health effects from ingesting 2,3,7,8-TCDD" (Fig. 1.1), exposure is measured in milligrams of 2,3,7,8-TCDD per kilogram of body weight (mg/kg). In all graphs, effects in animals are shown on the left side and effects in humans on the right side.

In the second set of graphs (Fig. 1.2), the same relationship is represented for the known "Health effects from skin contact with products containing 2,3,7,8-TCDD." Exposures again are measured in milligrams of 2,3,7,8-TCDD per kilogram of body weight (mg/kg).

The levels marked on Fig. 1.1, as anticipated to be associated with minimal risk for effects other than cancer in humans, are based on information from animal studies; therefore, some uncertainty still exists. For cancer, the U.S. Environmental Protection Agency (EPA) has estimated that lifetime exposure to 1 nanogram of 2,3,7,8-TCDD per kilogram per day would result in 1560 or 1,560,000 additional cases of cancer in a population of 10,000 or 10,000,000 people, respectively. It should be noted that these risk values are plausible upper-limit estimates. Actual risk levels are unlikely to be higher and may be lower. (One nanogram is one-billionth of a gram.)

There was not enough information to prepare a graph for exposure by breathing.

1.7 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

Both the EPA and the International Agency for Research on Cancer (IARC) have concluded that 2,3,7,8-TCDD causes cancer in animals, which suggests that 2,3,7,8-TCDD may cause cancer in humans.

The EPA calculated health advisories (HAs) for 2,3,7,8-TCDD in drinking water, that is, estimates of levels below which adverse health effects are not expected to occur. The 1-day HA is 0.000001 milligrams per liter (mg/L) (10 parts per trillion, ppt) for a child; the 10-day HA is 0.00000001 mg/L (0.001 ppt) for a child. The longer-term HA is 0.0000001 mg/L for a child and 0.000000035 mg/L for an adult (0.1 and 0.035 ppt, respectively); the lifetime HA is 0.000000035 mg/L for adults (0.035 ppt). The EPA also calculated the amount of 2,3,7,8-TCDD in ambient water (lakes and rivers) which would be associated with increases in one additional incidence of cancer over background cancer incidence in a population of 1,000,000 to be 0.000000000013 mg/L. This calculated measurement takes into account that 2,3,7,8-TCDD concentrates in fish; hence, exposure may occur through both the drinking of water and the eating of fish. The Federal Drug Administration has calculated that fish containing less than 25 ppt of 2,3,7,8-TCDD should pose no serious health concerns.

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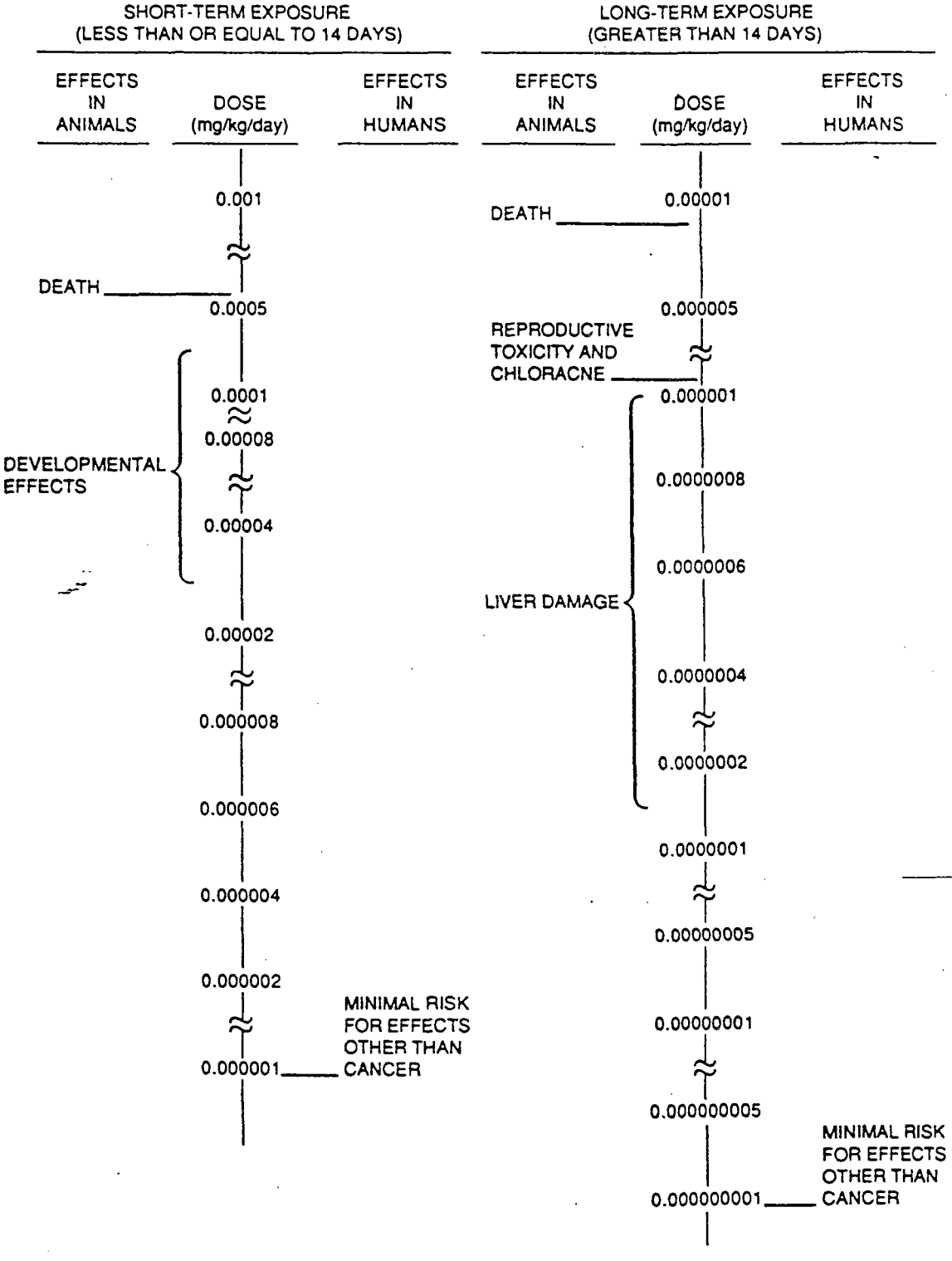


Fig. 1.1. Health effects from ingesting 2,3,7,8-TCDD.

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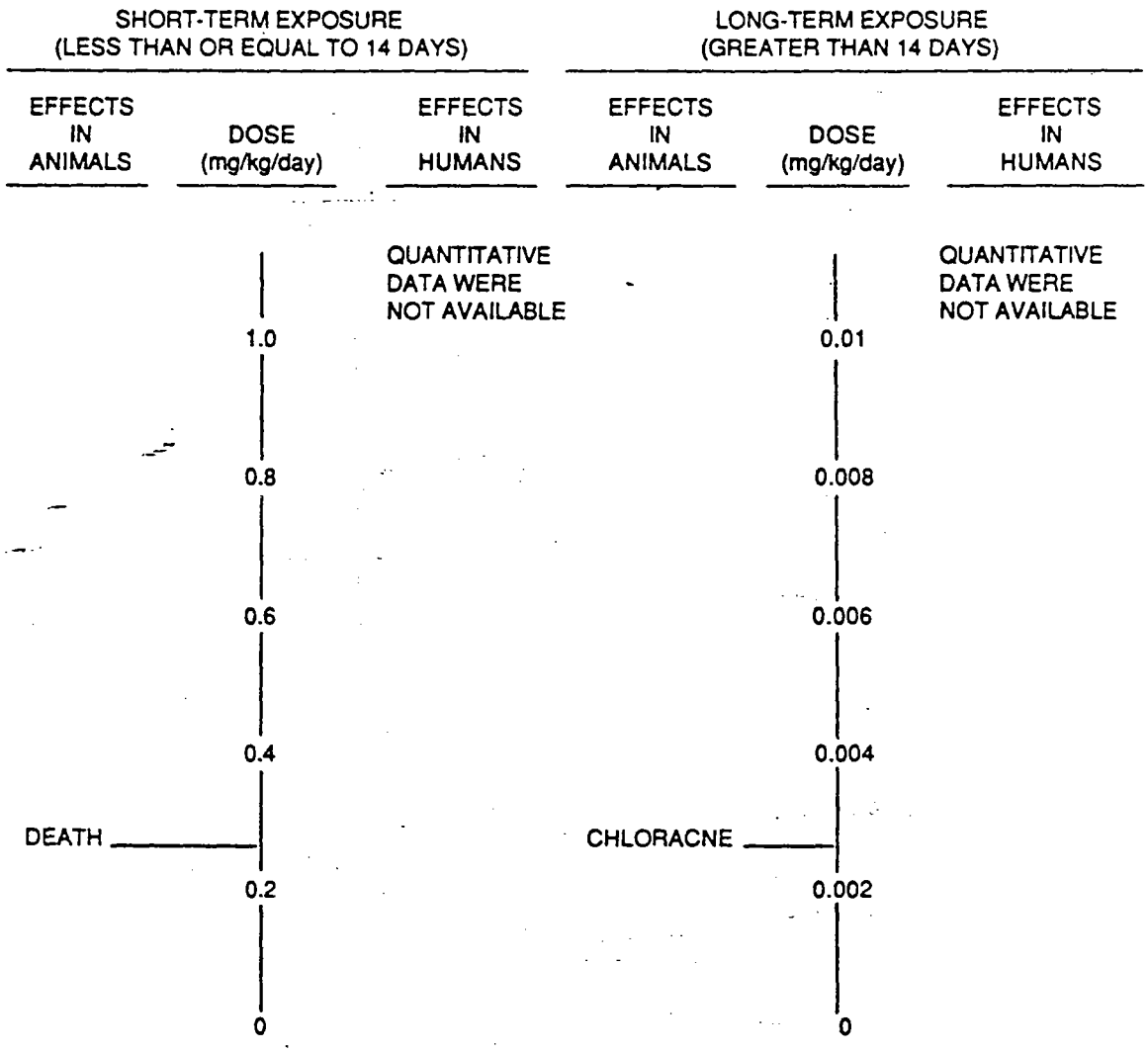


Fig. 1.2. Health effects from skin contact with 2,3,7,8-TCDD.

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2. HEALTH EFFECTS SUMMARY

2.1 INTRODUCTION

This section summarizes and graphs data on the health effects concerning exposure to 2,3,7,8-TCDD. The purpose of this section is to present levels of significant exposure for 2,3,7,8-TCDD based on key toxicological studies, epidemiological investigations, and environmental exposure data. The information presented in this section is critically evaluated and discussed in Sect. 4, Toxicological Data, and Sect. 7, Potential for Human Exposure.

This Health Effects Summary section comprises two major parts. Levels of Significant Exposure (Sect. 2.2) presents brief narratives and graphics for key studies in a manner that provides public health officials, physicians, and other interested individuals and groups with (1) an overall perspective of the toxicology of 2,3,7,8-TCDD and (2) a summarized depiction of significant exposure levels associated with various adverse health effects. This section also includes information on the levels of 2,3,7,8-TCDD that have been monitored in human fluids and tissues, and information about levels of 2,3,7,8-TCDD found in environmental media and their association with human exposures.

The significance of the exposure levels shown on the graphs may differ depending on the user's perspective. For example, physicians concerned with the interpretation of overt clinical findings in exposed persons or with the identification of persons with the potential to develop such disease may be interested in levels of exposure associated with frank effects (Frank Effect Level, FEL). Public health officials and project managers concerned with response actions at Superfund sites may want information on levels of exposure associated with more subtle effects in humans or animals (Lowest-Observed-Adverse-Effect Level, LOAEL) or exposure levels below which no adverse effects (No-Observed-Adverse-Effect Level, NOAEL) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels) are of interest to health professionals and citizens alike.

Adequacy of Database (Sect. 2.3) highlights the availability of key studies on exposure to 2,3,7,8-TCDD in the scientific literature and displays these data in three-dimensional graphs consistent with the format in Sect. 2.2. The purpose of this section is to suggest where there might be insufficient information to establish levels of significant human exposure. These areas will be considered by the Agency for Toxic Substances and Disease Registry (ATSDR), EPA, and the National Toxicology Program (NTP) of the U.S. Public Health Service in order to develop a research agenda to provide this information.

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2.2 LEVELS OF SIGNIFICANT EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the toxicology data summarized in this section are organized first by route of exposure--inhalation, ingestion, and dermal--and then by toxicological end points that are categorized into six general areas--lethality, systemic/target organ toxicity, developmental toxicity, reproductive toxicity, genetic toxicity, and carcinogenicity. The data are discussed in terms of three exposure periods--acute, intermediate, and chronic.

Two kinds of graphs are used to depict the data. The first type is a "thermometer" graph. It provides a graphical summary of the human and animal toxicological end points (and levels of exposure) for each exposure route for which data are available. The ordering of effects does not reflect the exposure duration or species of animal tested. The second kind of graph shows Levels of Significant Exposure (LSE) for each route and exposure duration. The points on the graph showing NOAELs and LOAELs reflect the actual doses (levels of exposure) used in the key studies. No adjustments for exposure duration or intermittent exposure protocol were made.

Adjustments reflecting the uncertainty of extrapolating animal data to man, intraspecies variations, and differences between experimental versus actual human exposure conditions were considered when estimates of levels posing minimal risk to human health were made for noncancer end points. These minimal risk levels were derived for the most sensitive noncancer end point for each exposure duration by applying uncertainty factors. These levels are shown on the graphs as a broken line starting from the actual dose (level of exposure) and ending with a concave-curved line at its terminus. Although methods have been established to derive these minimal risk levels (Barnes et al. 1987), shortcomings exist in the techniques that reduce the confidence in the projected estimates. Also shown on the graphs under the cancer end point are low level risks (10^{-4} to 10^{-7}) reported by EPA. In addition, the actual dose (level of exposure) associated with tumor incidence is plotted.

2.2.1 Key Studies and Graphical Presentations

It is difficult to assess the risk to humans from exposure to 2,3,7,8-TCDD. There are many species differences in toxicity; the monkey and guinea pig are apparently the most sensitive, and the hamster is the least sensitive. It is not known how sensitive humans are to 2,3,7,8-TCDD. In addition, 2,3,7,8-TCDD strongly adsorbs to materials such as soil, which may significantly affect the bioavailability and toxicity of this compound. Animal studies have generally been conducted with 2,3,7,8-TCDD administered in oily vehicles from which the compound is readily bioavailable. There are insufficient data available to consider the effect of bioavailability on the toxicity studies used to define the risk from exposure to 2,3,7,8-TCDD. Both bioavailability and species differences in sensitivity should be considered when evaluating the remainder of the data presented.

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Oral and dermal NOAELs and LOAELs are presented on "thermometer" graphs in Figs. 2.1 and 2.2, respectively. Although some qualitative data are available for humans, quantitative data were insufficient to graphically present inhalation effects.

Levels of significant exposure are depicted in Figs. 2.3 and 2.4 for the oral and dermal routes. There were insufficient human and animal inhalation data for graphical representation. The intermediate minimal risk level for oral exposure was calculated by EPA (1985a) from a three-generation reproductive toxicity study in the rat, with effects observed in the fetuses. Since the effects were attributed to in utero fetal exposure, which chronologically represents an exposure of relatively short duration, as well as the chronic exposure of the dams, the same minimal risk level for chronic exposure was calculated from these data.

2.2.1.1 Inhalation

No studies are available on the inhalation toxicity of 2,3,7,8-TCDD. Exposure through inhalation, however, may also have occurred to the population exposed to chemicals contaminated with TCDD in accidental releases or in the workplace (especially herbicide spraying).

2.2.1.2 Oral

Lethality and decreased longevity. There have been no reports of death in humans as a result of oral exposure to 2,3,7,8-TCDD.

2,3,7,8-TCDD is highly toxic to all mammalian species, even though there is a large difference in species sensitivity. LD_{50} values range from 0.6 μg/kg in male guinea pigs (Schwetz et al. 1973) to 5500 μg/kg in hamsters (Henck et al. 1981). These values are plotted in Figs. 2.1 and 2.3 for the lethality of acute oral exposure. Death usually occurs 13 to 18 days after a single exposure. Extended exposure in a 90-day feeding study in guinea pigs resulted in an estimated 50% mortality after consumption of a total of 0.8 μg of 2,3,7,8-TCDD/kg (0.008 μg/kg/day) (DeCaprio et al. 1986), whereas deaths did not occur at 0.0006 μg/kg/day (NOAEL). Five of eight female monkeys that ingested approximately 0.01 μg of 2,3,7,8-TCDD/kg/day for 9 months died (Allen et al. 1977). These FELs in guinea pigs and monkeys and the NOAEL in guinea pigs are shown in Figs. 2.1 and 2.3.

Target organ/systemic toxicity. Four major toxic effects characteristic of 2,3,7,8-TCDD are chloracne, the wasting syndrome, hepatotoxicity, and immunotoxicity. Chloracne, immunotoxicity, hyperpigmentation, hyperkeratosis, hirsutism of the skin, possible hepatotoxicity, hypertriglycerid and hypercholesterolemia, aching muscles, loss of appetite, weight loss, digestive disorders, headaches, neuropathy, insomnia, sensory changes, and loss of libido have been observed in humans exposed to chemicals contaminated with 2,3,7,8-TCDD. Because some herbicides and some industrial chemicals contain 2,3,7,8-TCDD as a contaminant, the primary route of exposure is most likely to be dermal, although some oral and inhalation exposure probably also occurs.

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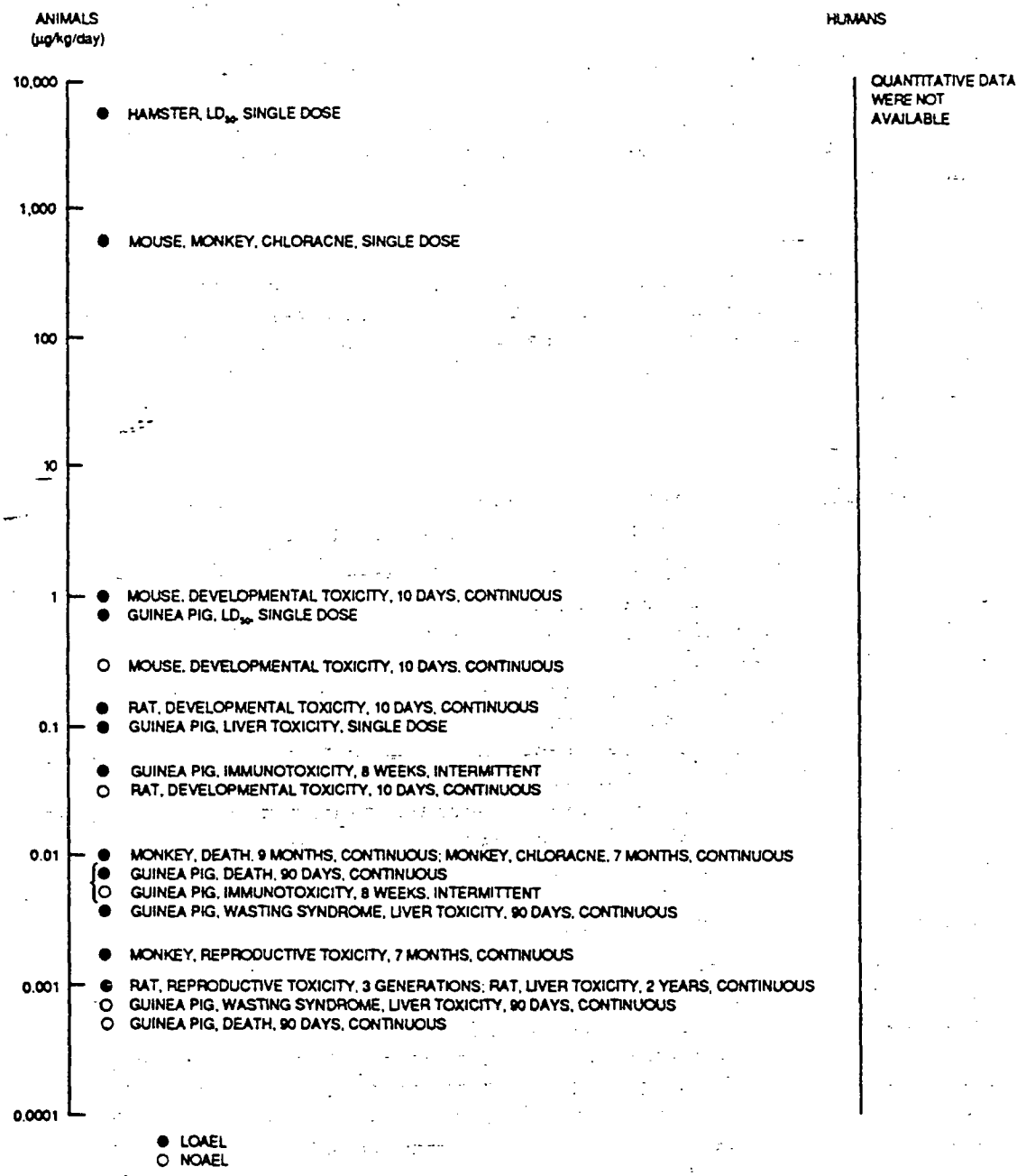


Fig. 2.1. Effects of 2,3,7,8-TCDD—oral exposure.

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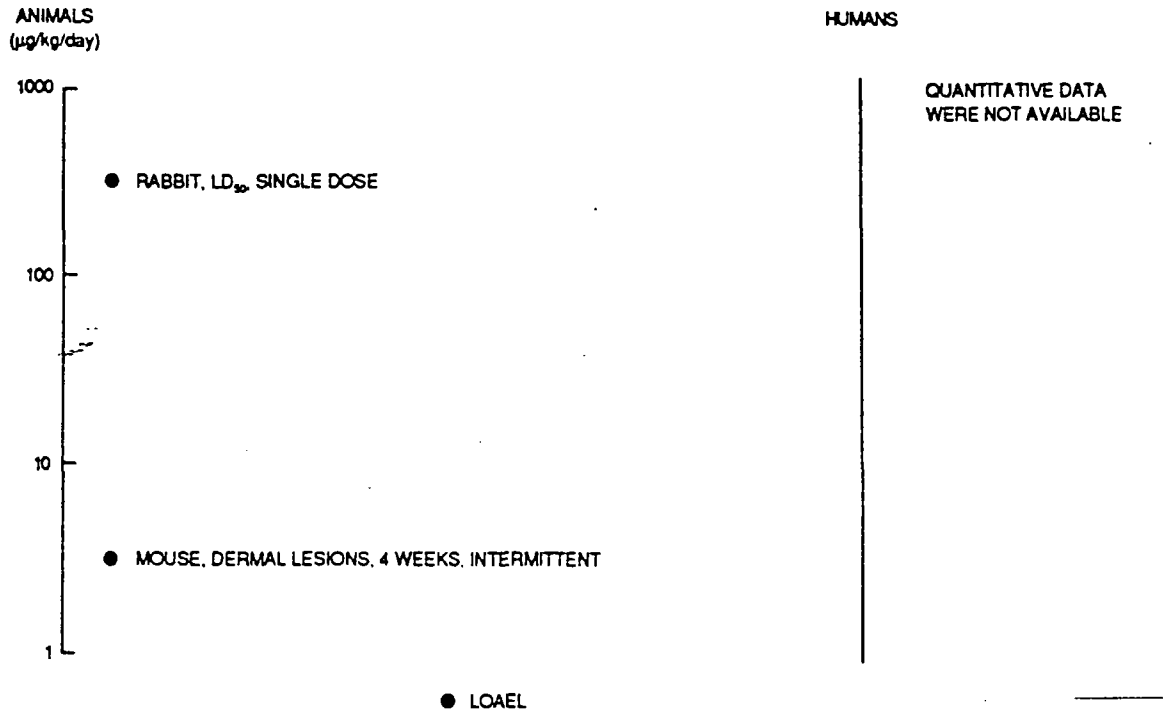


Fig. 2.2. Effects of 2,3,7,8-TCDD—dermal exposure.

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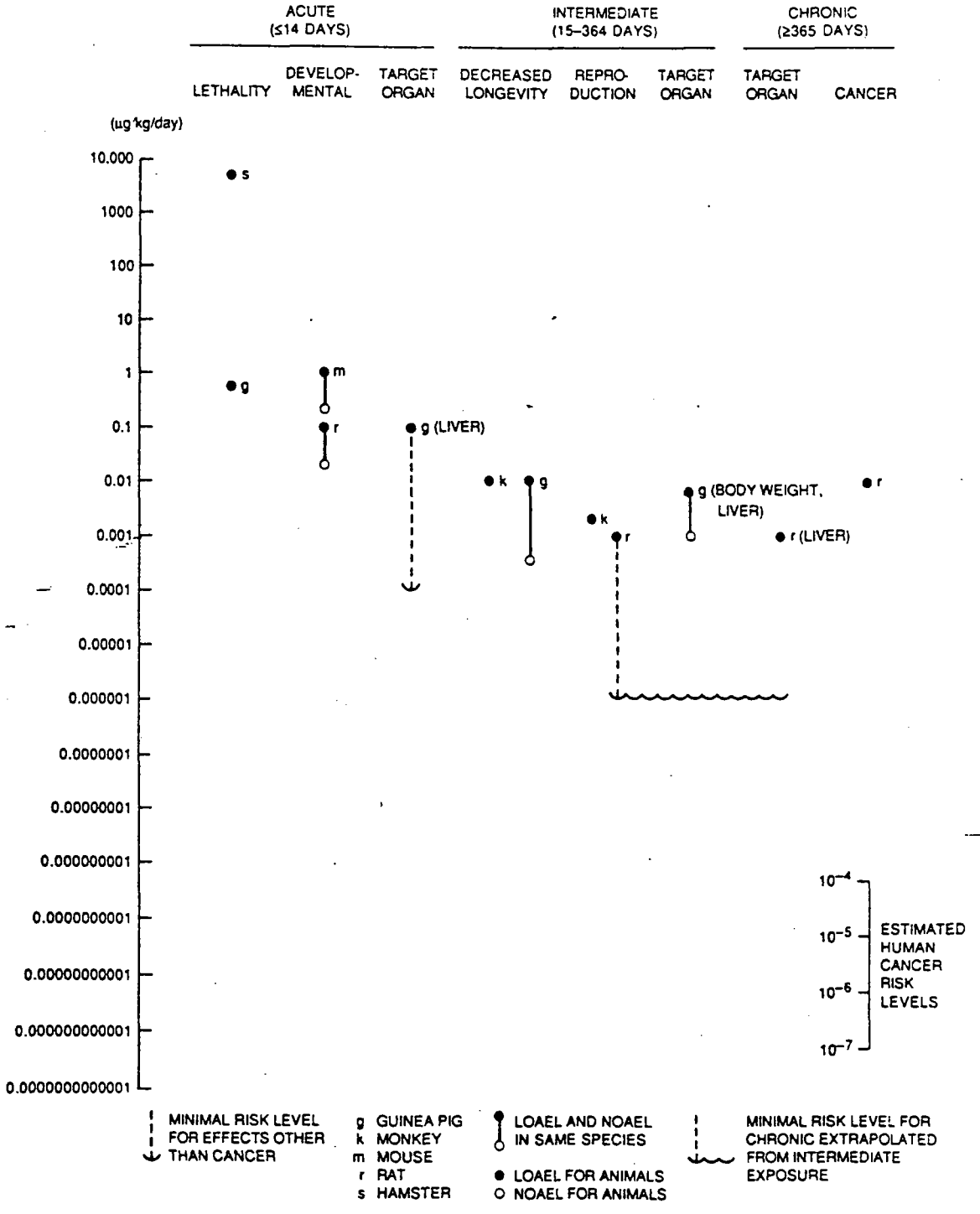


Fig. 2.3. Levels of significant exposure for 2,3,7,8-TCDD—oral.

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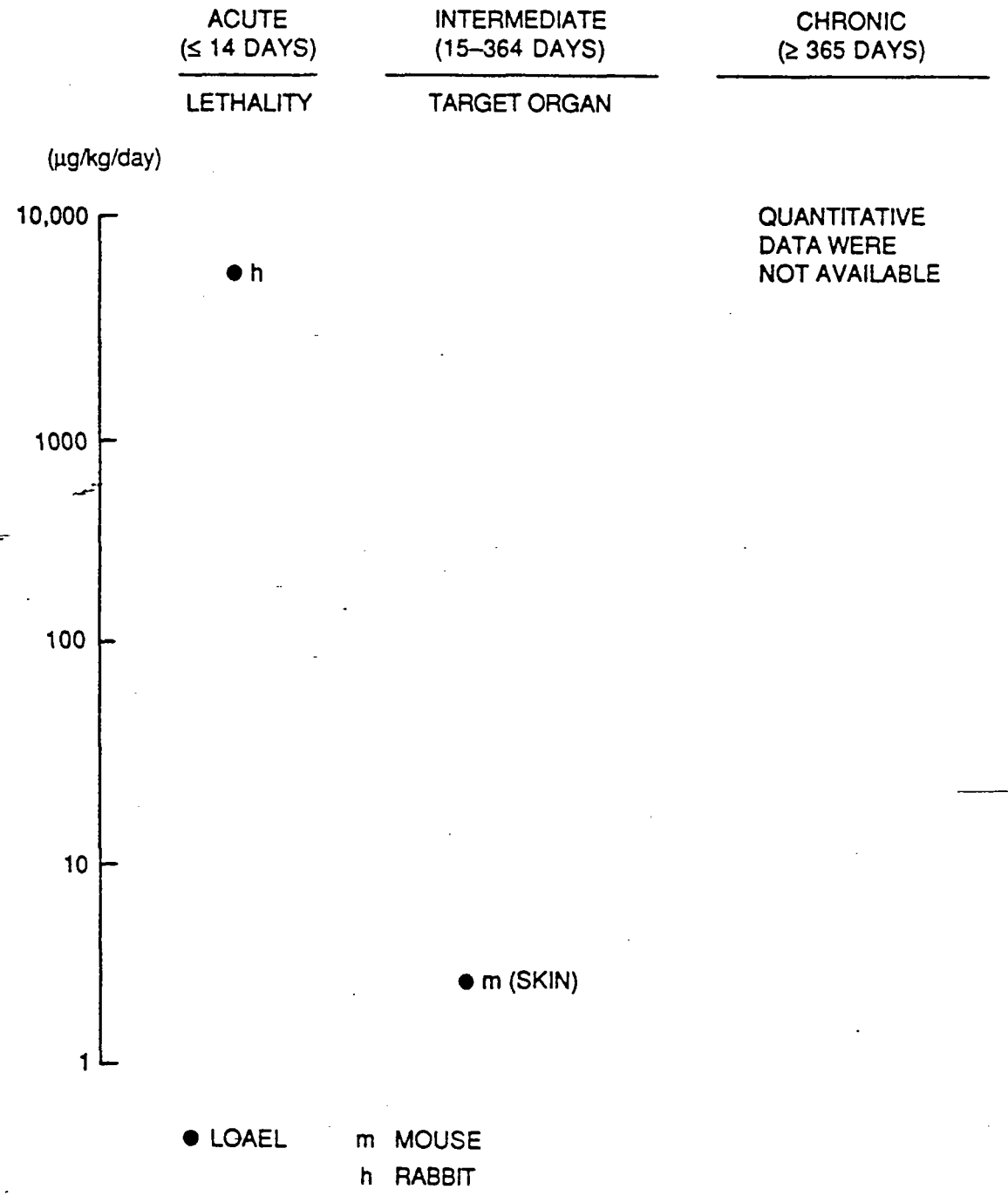


Fig. 2.4. Levels of significant exposure for 2,3,7,8-TCDD—dermal.

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Since chloroacne, the only lesion definitively identified in humans as resulting from 2,3,7,8-TCDD exposure, can only be detected in a few species, the investigation of this effect has been limited. In hairless mice, chloracne was produced after a single dose of 2,3,7,8-TCDD at 70 μg/kg (Greig 1984), whereas a single dose of 70 μg/kg, or a dose of ~0.01 μg/kg/day in a 7-month feeding study in monkeys, produced similar lesions on the face (McConnell et al. 1978, Allen et al. 1977). These data are indicated in Fig. 2.1. The available studies were not designed to define a dose-response relationship; only FELs are reported (see Fig. 2.1).

The wasting syndrome is characterized by extreme loss of body weight. In acute studies, this syndrome is associated with lethal doses. A dose-response relationship for the wasting syndrome has been defined in a 90-day study in guinea pigs (DeCaprio et al. 1986). Female Hartley guinea pigs were maintained on diets providing average 2,3,7,8-TCDD doses of 0, 0.12, 0.68, 4.86, and 31 ng/kg/day. The high dose represented a FEL; a 40% decrease in body weight and death occurred. The 4.86-ng/kg (0.005 μg/kg) dose represented a LOAEL, with a 13% decrease in body weight and no mortality. The 0.68 ng/kg (0.0007 μg/kg) is a NOAEL for this effect. The LOAEL and NOAEL for intermediate exposure are indicated in Figs. 2.1 and 2.3.

2,3,7,8-TCDD is hepatotoxic in all species tested; however, the severity of the lesions depends on the species studied. Although liver damage is not as severe in the guinea pig, the most sensitive species tested with regard to lethality, liver changes such as focal necrosis and hypertrophy have been observed at very low doses by Turner and Collins (1983). In this study, a small group of male and female guinea pigs was given a single dose of 2,3,7,8-TCDD at 0.1, 0.5, 2.5, 12.5, or 20 μg/kg. Effects on the liver occurred in all groups. The low dose was considered a LOAEL (see Figs. 2.1 and 2.3), but a NOAEL was not available. A minimal risk for effects of acute oral exposure (see Fig. 2.3) was calculated from this LOAEL by EPA (1985a), since it was the most sensitive end point in acute studies. Similar liver damage was observed in guinea pigs in the 90-day feeding study by DeCaprio et al. (1986). The NOAEL was 0.68 ng/kg and the LOAEL was 4.86 ng/kg, the same as the NOAEL and LOAEL for the wasting syndrome (see Figs. 2.1 and 2.3). There are no chronic studies in guinea pigs; however, in chronic studies in rats, Kociba et al. (1978a,b) and NTP (1982a) reported "toxic hepatitis" and degenerative changes at the lowest dietary exposure that provided a dose of 0.001 μg of 2,3,7,8-TCDD/kg/day in these 2-year studies. Again, only a LOAEL, which is plotted in Figs. 2.1 and 2.3, is available for this end point after chronic exposure.

The guinea pig also appears to be the most sensitive species to the immunotoxic effects of 2,3,7,8-TCDD. Vos et al. (1973) observed a decrease in thymus weight, total lymphocyte number, and total leukocyte number in groups of 10 guinea pigs given 2,3,7,8-TCDD weekly for 8 weeks at doses of 0, 0.008, 0.04, or 0.2 μg/kg. The high dose was a FEL and also produced other toxic effects including loss of body weight. A LOAEL of 0.04 μg/kg and a NOAEL of 0.008 μg/kg were defined. At the LOAEL, body weight was comparable to controls. The LOAEL and NOAEL for immunotoxicity are plotted on Fig. 2.1, but not on Fig. 2.3 because liver toxicity and wasting syndrome are more sensitive end points of

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intermediate oral exposure in guinea pigs. Immunotoxicity is not limited to guinea pigs; Thigpen et al. (1975) reported that mice given 4 weekly exposures to 2,3,7,8-TCDD at doses as low as 1 μg/kg were more sensitive to *Salmonella*-induced death. This dose caused no gross signs of toxicity in animals not exposed to *Salmonella*.

Developmental toxicity. There have been no reports of developmental toxicity in humans as a result of oral exposure to 2,3,7,8-TCDD.

2,3,7,8-TCDD produces anomalies in the fetus, including cleft palate and hydronephrotic kidneys in mice and internal organ hemorrhage in the rat. FELs in rats were reported to be 0.125 μg/kg/day after administration of the compound on days 6 through 15 of gestation (Sparschu et al. 1971a,b), whereas the next lower dose tested, 0.03 μg/kg, was a NOAEL (see Figs. 2.1 and 2.3). Doses of 1 μg/kg caused fetal death. Although developmental effects are also observed in mice, this species appears less sensitive, with FELs of ~1 μg/kg/day (when administered during organogenesis) and a NOAEL of ~0.3 μg/kg (Neubert and Dillman 1972) (see Figs. 2.1 and 2.3).

Reproductive toxicity. There have been no reports of reproductive toxicity in humans as a result of oral exposure to 2,3,7,8-TCDD.

Murray et al. (1979) conducted a three-generation reproductive toxicity study in rats. 2,3,7,8-TCDD was administered in the diet at levels that provided doses of 0.001, 0.01, and 0.1 μg/kg/day. The high dose resulted in decreased fetal survival. Murray et al. (1979) concluded that the 0.01-dose represented a LOAEL (with effects observed on litter size and fetal and neonatal survival) and that the 0.001-dose was considered to be a NOAEL. Nisbet and Paxton (1982) reevaluated the above data, using different statistical methods, and concluded that the lowest dose tested produced dilated renal pelvises, decreased fetal weight, and changes in the gestational index, which indicated that 0.001 μg/kg was in fact a LOAEL (see Figs. 2.1 and 2.3). EPA (1985a) calculated a minimal risk for intermediate and chronic oral exposure from the Nisbet and Paxton (1982) analysis (see Fig. 2.3).

In a study with monkeys maintained on a diet for 7 months, which provided 2,3,7,8-TCDD at levels of 0.0015 and 0.01 μg/kg/day, there were spontaneous abortions in two-thirds of the monkeys at both dose levels (Allen et al. 1979). The 0.0015-μg/kg/day level is indicated on Figs. 2.1 and 2.3. This study, which reported severe frank effects, indicates that monkeys may be the most sensitive species with regard to the reproductive toxicity of 2,3,7,8-TCDD. This study, however, only provided FELs, and additional data were not available for determining a NOAEL or the actual relationship between species sensitivity.

Genotoxicity. There have been no reports of genotoxicity in humans as a result of oral exposure to 2,3,7,8-TCDD.

2,3,7,8-TCDD has produced mostly negative responses in tests for genotoxicity; however, there are a few positive responses, which may suggest that 2,3,7,8-TCDD is genotoxic (see Sect. 4.3.5 on genotoxicity). Some of the inconsistencies observed may be related to experimental difficulties in testing 2,3,7,8-TCDD, such as the very low solubility of this compound and the high toxicity in vivo (which limits

the quantity that can be tested), rather than to inherent biological inactivity.

Carcinogenicity. There have been no reports of increased cancer incidence in humans as a result of oral exposure to 2,3,7,8-TCDD.

2,3,7,8-TCDD has been demonstrated to be an animal carcinogen in both rats and mice in an NTP (1982a) bioassay, and in rats in a 2-year bioassay by Kociba et al. (1978a,b). EPA (1985a) used female rat data from the Kociba et al. (1978a,b) study to derive a q_1^*. In this derivation, the total incidences for tumors of the liver, lung, hard palate, or nasal turbinates, as reported by Kociba et al. (1978a,b), were combined. These data, along with a similar set of data derived for EPA by Squire on the reevaluation of the histologic section from the Kociba et al. (1978a,b) study, were used by EPA (1985a) to derive a q_1^*. The tumor incidences reported by Kociba et al. (1978a,b) for doses of 0, 0.001, 0.01, and 0.1 μg/kg/day were 9/85, 3/48, 18/48, and 34/40, respectively, whereas the respective values reported by Squire were 16/85, 8/48, 27/48, and 34/40. The lower dose of 0.01 μg/kg/day, associated with increased tumor incidence, is indicated on Fig. 2.3. The q_1^* thus calculated was 1.56×10^5 $(mg/kg/day)^{-1}$. For cancer, EPA has estimated that for a population of 10,000 people exposed to 0.6 pg/kg/day, the cancer risk is not likely to exceed 1/10,000, and similarly, that for a lesser exposure of 0.0006 pg/kg/day to 10,000,000 people, the expected cancer risk would not exceed 1/10,000,000 (Fig. 2.3).

Lethality and decreased longevity. There have been no reports of death in humans as a result of dermal exposure to 2,3,7,8-TCDD.

Schwetz et al. (1973) reported a dermal LD_{50} value of 275 μg/kg in rabbits. As in oral studies, there was a protracted length of time between application and death. No other data were available. The LD_{50} is plotted on Figs. 2.2 and 2.4.

Target organ/systemic toxicity. Chloracne is the only substantiated effect in humans produced by certain compounds contaminated with 2,3,7,8-TCDD. As reviewed by Taylor (1979) and Suskind (1985), these persistent, deforming face and upper body lesions have been recognized for many years as resulting from exposure to certain halogenated aromatic compounds, and it is believed that 2,3,7,8-TCDD is the most effective compound in producing this lesion. There is, however, no information on the levels of exposure needed to produce chloracne in humans, and, thus, quantitative risk assessment cannot be performed.

In addition, there are data that suggest that 2,3,7,8-TCDD is hepatotoxic in humans. In populations exposed to herbicides and other industrial chemicals contaminated with 2,3,7,8-TCDD, there have been reports of increased serum levels of liver enzymes and the development of porphyria cutanea tarda (EPA 1985a). In all studies, however, exposure may have been to chemicals that also could cause liver damage, and as pointed out by Jones and Chelsky (1986), the diagnosis of porphyria cutanea tarda in some of the studies may be questionable. It is thus difficult to assert that the presumed exposure to 2,3,7,8-TCDD resulted in liver injury, and even if 2,3,7,8-TCDD induced liver damage,

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there are no human data available that could provide dose-response information.

The only animal data that provide quantitative information on chloracne are provided by the study of Puhvel et al. (1982), in which hairless mice given 0.1 μg of 2,3,7,8-TCDD per application three times per week for 4 weeks developed dermal lesions that resembled some features of chloracne in humans. Assuming that a mouse weighs 0.03 kg, the dose is 3.3 μg/kg. This study, however, only used one dose which was a FEL; hence, it does not provide the necessary information for defining a dose-response relationship. The dose is plotted on Figs. 2.2 and 2.4 for intermediate target organ toxicity of dermal exposure.

Developmental toxicity. Studies of human populations exposed to herbicides and other industrial chemicals contaminated with 2,3,7,8-TCDD have suggested that 2,3,7,8-TCDD produces a variety of developmental effects (Hanify et al. 1981, Nelson et al. 1979, McQueen et al. 1977, Smith et al. 1982). After reviewing these studies, EPA (1985a) indicated that the data were not inconsistent with 2,3,7,8-TCDD adversely affecting development, but as a result of the limitations of the data, these studies could not prove an association with 2,3,7,8-TCDD exposure and the observed effect. The major limitations in these human studies were the concomitant exposure to other potentially toxic chemicals and the lack of any specific quantitative data on the extent of exposure of individuals within the study group.

No animal studies were available on the developmental toxicity of 2,3,7,8-TCDD following dermal exposure.

Reproductive toxicity. EPA (1985a) has reviewed human reproductive toxicity studies of groups exposed to herbicides and other industrial chemicals contaminated with 2,3,7,8-TCDD (EPA 1979, Field and Kerr 1979, Nelson et al. 1979, Thomas 1980, Dept. of Health New Zealand 1980, McQueen et al. 1977, Aldred 1978, Smith et al. 1982, Bonaccorsi et al. 1978, Reggiani 1980, Bisanti et al. 1980). These studies did not provide a scientifically valid indication that 2,3,7,8-TCDD adversely affects either male or female reproductive performance, or that exposure to 2,3,7,8-TCDD is without effect. The limitations of the studies are similar to those discussed in the section above.

No animal studies were available on the reproductive toxicity of 2,3,7,8-TCDD following dermal exposure.

Carcinogenicity. EPA (1985a) also reviewed a series of epidemiology studies of populations exposed to herbicides and other industrial chemicals contaminated with 2,3,7,8-TCDD. Some of these studies indicated an association of exposure with the development of soft tissue sarcomas at a variety of sites and an increase in non-Hodgkins lymphomas (Axelson et al. 1980; Theiss and Frentzel-Beyme 1977; Hardell and Sandstrom 1979; Eriksson et al. 1979, 1981; Hardell et al. 1980, 1981; Lynge 1985; Putoni et al. 1986). These studies also have the same limitations as those described above, as well as additional questions on whether tumors of various sites should have been grouped together for the analyses. There are also a number of epidemiology studies of populations with exposures to contaminated herbicides and industrial chemicals similar to those above where an elevated incidence

of cancer was not detected (Ott et al. 1980, 1987; Zack and Suskind 1980; Cook et al. 1980, 1985, 1986; Cook 1981; Pazderova-Vejlupkova et al. 1981; Johnson et al. 1981; Riihimaki et al. 1982; Smith et al. 1983; Wolfe et al. 1984; Smith and Pearce 1985; Fingerhut et al. 1984). Besides the confounding factors previously described regarding epidemiological studies of 2,3,7,8-TCDD, the studies that failed to demonstrate an association between cancer and exposure were additionally confounded by the lack of a sufficient time period to allow for the latency of tumor development, the apparent lack of a control population with no known exposure to 2,3,7,8-TCDD (as indicated by the analysis of fat biopsies, the general population has some exposure), and inconclusive data on the actual levels of exposure in the exposed population. It was again concluded that the human data could neither prove nor disprove that 2,3,7,8-TCDD is a human carcinogen.

There are some data from experimental animal studies regarding the dermal carcinogenicity of 2,3,7,8-TCDD. In Swiss mice, females, but not males, developed skin tumors following dermal application of 2,3,7,8-TCDD at 0.01 μg per application three times per week (NTP 1982b). There has also been mixed evidence that 2,3,7,8-TCDD is a tumor promotor. Poland et al. (1982) observed tumor-promoting activity in hairless mice, but not in mice heterozygous for the hairless trait. Similarly, Berry et al. (1978), Slaga and Nesnows (1985), and NTP (1982) have not been able to demonstrate promoting activity in CD-1, Sencar, or Swiss-Webster mice.

2.2.2. Biological Monitoring as a Measure of Exposure and Effects

There are no adequate tests or analyses of biological samples that could indicate the extent of exposure to 2,3,7,8-TCDD; however, some qualitative indications can be obtained. Adipose tissue has been shown to be one of the primary storage sites for 2,3,7,8-TCDD, and tissue samples have been analyzed, although mixed results have been obtained. As reported by Nygren et al. (1986), Young et al. (1983) failed to detect elevated levels of 2,3,7,8-TCDD in the adipose tissues of Vietnam veterans exposed to Agent Orange, whereas Gross et al. (1984) detected increased levels in veterans who had been exposed to high levels of Agent Orange. As reported by Nygren et al. (1986), higher levels of 2,3,7,8-TCDD in adipose tissue have also been reported in individuals exposed during a transformer fire accident in Binghamton, New York, or the accident in Seveso, Italy. In another study, 39 exposed individuals in Missouri had median adipose 2,3,7,8-TCDD levels of 17 ppt, with a range of 2.8 to 750 ppt, whereas the unexposed control group had a median level of 6.4 and a range of 1.4 to 20 ppt (Patterson et al. 1986). Although six of the subjects in the exposed group had levels >5 times higher than the highest control, there was also extensive overlap between the groups.

Although the monitoring of adipose tissue may provide some qualitative indication that exposure has occurred, there are no good correlations available between adipose tissue levels and the extent of exposure. In addition, the background level of 2,3,7,8-TCDD in the adipose tissue of individuals with no known history of exposure to 2,3,7,8-TCDD generally are in the range of 5 to 18 ppt. This would suggest an ubiquitous exposure to 2,3,7,8-TCDD, which makes it difficult

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to assess the contribution to body burden from any particular small additional exposure. A similar lack of correlation between estimated exposure to 2,3,7,8-TCDD and sera levels of 2,3,7,8-TCDD was reported in a preliminary study in the MMWR (1987), in which Vietnam veterans with military histories indicating exposure to herbicides containing 2,3,7,8-TCDD were compared to non-Vietnam veterans with presumably no unusual exposure to 2,3,7,8-TCDD. In these preliminary results, at least, there was no difference in the range of 2,3,7,8-TCDD levels (1 to 9 ppt based on lipid weight) or the median 2,3,7,8-TCDD level (3.9 ppt for the presumably exposed group and 3.8 ppt for the nonexposed group). Biological monitoring, such as monitoring levels in breast milk, only provides possible qualitative indications of exposure. With commonly available analytical techniques, 2,3,7,8-TCDD is not detected in body fluids, such as blood or urine, although a recent method with parts-per-quadrillion sensitivity has detected 2,3,7,8-TCDD in human serum (Patterson et al. 1987b).

There are also no clear tests for the effects of exposure to 2,3,7,8-TCDD. Chloracne is the only effect that is clearly associated with exposure to chemicals contaminated with 2,3,7,8-TCDD; however, chloracne is also caused by other halogenated aromatic compounds. The development of chloracne in an individual who may have been exposed to 2,3,7,8-TCDD would provide supportive evidence that exposure to this chemical had occurred. The development of chloracne, however, does not indicate the extent of exposure. Likewise, the data of Hoffman et al. (1986) suggest that the development of immunotoxicity would provide supportive evidence for exposure to 2,3,7,8-TCDD; however, again, the extent of exposure is not indicated. In addition, there are insufficient data to state with certainty that immunotoxicity is associated with 2,3,7,8-TCDD exposure. Other signs of toxicity observed in animal studies (i.e., liver damage, effects on lipid metabolism, and types of circulating lipids) have not been demonstrated in humans and are not useful in determining that exposure to 2,3,7,8-TCDD has occurred.

2.2.3 Environmental Levels as Indicators of Exposure and Effects

2.2.3.1 Levels found in the environment

There are little data to associate environmental levels with significant human exposure. 2,3,7,8-TCDD has been monitored in the areas of extensive herbicide use and areas contaminated with 2,3,7,8-TCDD through industrial accidents; however, epidemiologic studies of inhabitants of these areas have lacked adequate exposure data that would permit the demonstration of a clear association between exposure and effects. The biological half-life of 2,3,7,8-TCDD in humans, calculated to be 5 years by Poiger and Schlatter (1986), would indicate that repeated exposure to low levels of 2,3,7,8-TCDD could substantially elevate the body burden of this compound to a level equivalent to the level obtained after a single exposure to a high level. Given the long half-life of 2,3,7,8-TCDD, the total exposure history of an individual has to be taken into account; hence, the environmental levels during a single particular exposure scenario may be misleading with regards to either effects observed or levels of body burden. The difficulties of estimating safe environmental levels have been discussed by Kimbrough et

al. (1984), who concluded that >1 ppb of 2,3,7,8-TCDD are levels of concern in residential soil.

2.2.3.2 Human exposure potential

It is not possible to state with any precision which route of exposure to 2,3,7,8-TCDD is most relevant to the human population. As with many human exposure scenarios, it is anticipated that all routes of exposure occur, although there are no data available to quantify the relative contribution of each route. It is anticipated that a significant route of exposure is dermal. Dermal exposure can occur through direct dermal contact with the mist of liquid chemical formulations of a herbicide containing 2,3,7,8-TCDD as a contaminant, or as a result of the long environmental half-life of 2,3,7,8-TCDD, by contact with soil or foliage (soon after contamination) in areas sprayed with these herbicides or contaminated through industrial accidents or waste disposal. In contaminated areas, exposure may also occur through ingestion or inhalation of soil or dust containing adsorbed 2,3,7,8-TCDD. Inhalation may be of particular concern where contaminated soils are being excavated or dust is being formed by other activities. In a 5-month clean-up operation of a 2,3,7,8-TCDD-contaminated Superfund site in Missouri, where ~6 in. of soil was removed with a backhoe from a 5000-ft^2 area, average 2,3,7,8-TCDD levels in the air were between 1 and 1.5 pg/m^3 (Fairless et al. 1987). 2,3,7,8-TCDD from industrial and municipal incinerators may also represent a source of long-term, low-level inhalation exposure. Exposure from ingesting contaminated food is also variable, with certain foods such as fish, which are known to concentrate 2,3,7,8-TCDD, providing potentially relatively high exposures.

Based on data on dermal and oral absorption in animals, it is anticipated that adsorbed 2,3,7,8-TCDD will not be as bioavailable as the 2,3,7,8-TCDD used in experimental studies and administered in oily vehicles. In the studies available, oral absorption of 2,3,7,8-TCDD adsorbed to soil was still substantial but only 50% of that from corn oil (absorption of 50 to 80%) (McConnell et al. 1984, Lucier et al. 1986, Umbreit et al. 1986a). Bioavailability also varies with the type of soil, as demonstrated by Umbriet et al. (1986b) for New Jersey and Missouri soils, where bioavailability from New Jersey soil was less than that from Missouri soils. The limited studies available may not be representative of the variation in bioavailability, since Poiger and Schlatter (1980) demonstrated that strong binding vehicles, such as activated carbon, can apparently reduce bioavailability to zero, and Philippi et al. (1981) and Huetter and Philippi (1982) demonstrated that the strength of adsorption increases with contact time in soil. Hence, factors such as soil type and contact time may affect the bioavailability of 2,3,7,8-TCDD.

It is likely that many of the same physical properties which result in strong binding to soil, such as extremely low water solubility and planar configuration, also result in very high bioconcentration factors. Because of the high lipophilicity and long half-life of 2,3,7,8-TCDD, exposure through ingestion of fatty tissues of fish that inhabit contaminated areas is anticipated to be significant. In addition, as a result of the lipophilic nature of milk, secretion of milk can provide a

relatively efficient mechanism for decreasing the body burden of 2,3,7,8-TCDD in females. As discussed by Graham et al. (1986), this elimination of 2,3,7,8-TCDD through mother's milk can result in large exposures of the infant. Since both milk and the fatty tissues of fish are essentially providing an oily vehicle, it seems likely that these sources would provide 2,3,7,8-TCDD in a form that is readily bioavailable.

2.3 ADEQUACY OF DATABASE

2.3.1 Introduction

Section 110 (3) of SARA directs the Administrator of ATSDR to prepare a toxicological profile for each of the 100 most significant hazardous substances found at facilities on the CERCLA National Priorities List. Each profile must include the following content:

"(A) An examination, summary, and interpretation of available toxicological information and epidemiologic evaluations on the hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects.

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure which present a significant risk to human health of acute, subacute, and chronic health effects.

(C) Where appropriate, an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans."

This section identifies data gaps in current knowledge relevant to developing levels of significant exposure for 2,3,7,8-TCDD. Such gaps are identified for certain health effects end points (lethality, system/target organ toxicity, developmental toxicity, reproductive toxicity, and cancer) reviewed in Sect. 2.2 of this profile in developing levels of significant exposure for 2,3,7,8-TCDD, and for other areas such as human biological monitoring and mechanisms of toxicity. The present section briefly summarizes the adequacy of existing human and animal data, identifies data gaps, and summarizes research in progress that may fill such gaps.

Specific research programs for obtaining data needed to develop levels of significant exposure for 2,3,7,8-TCDD will be developed by ATSDR, NTP, and EPA in the future.

2.3.2 Adequacy of the Database for Health Effect End Points

2.3.2.1 Introduction and graphic summary

The adequacy of the 2,3,7,8-TCDD database for health effect end points in humans and animals is depicted on bar graphs in Figs. 2.5 and 2.6, respectively.

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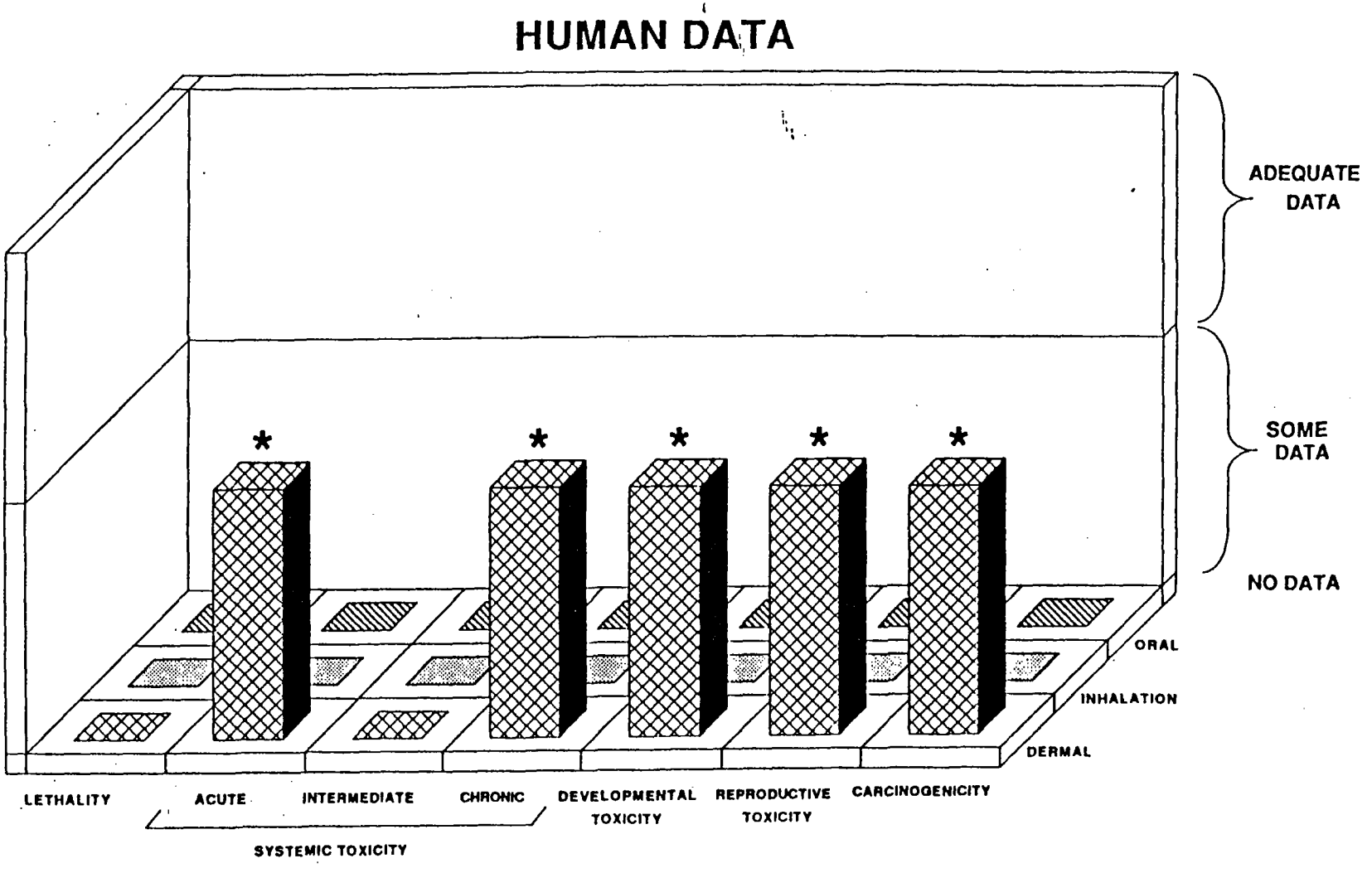


Fig. 2.5. Adequacy of the database on health effects of 2,3,7,8-TCDD (human data).

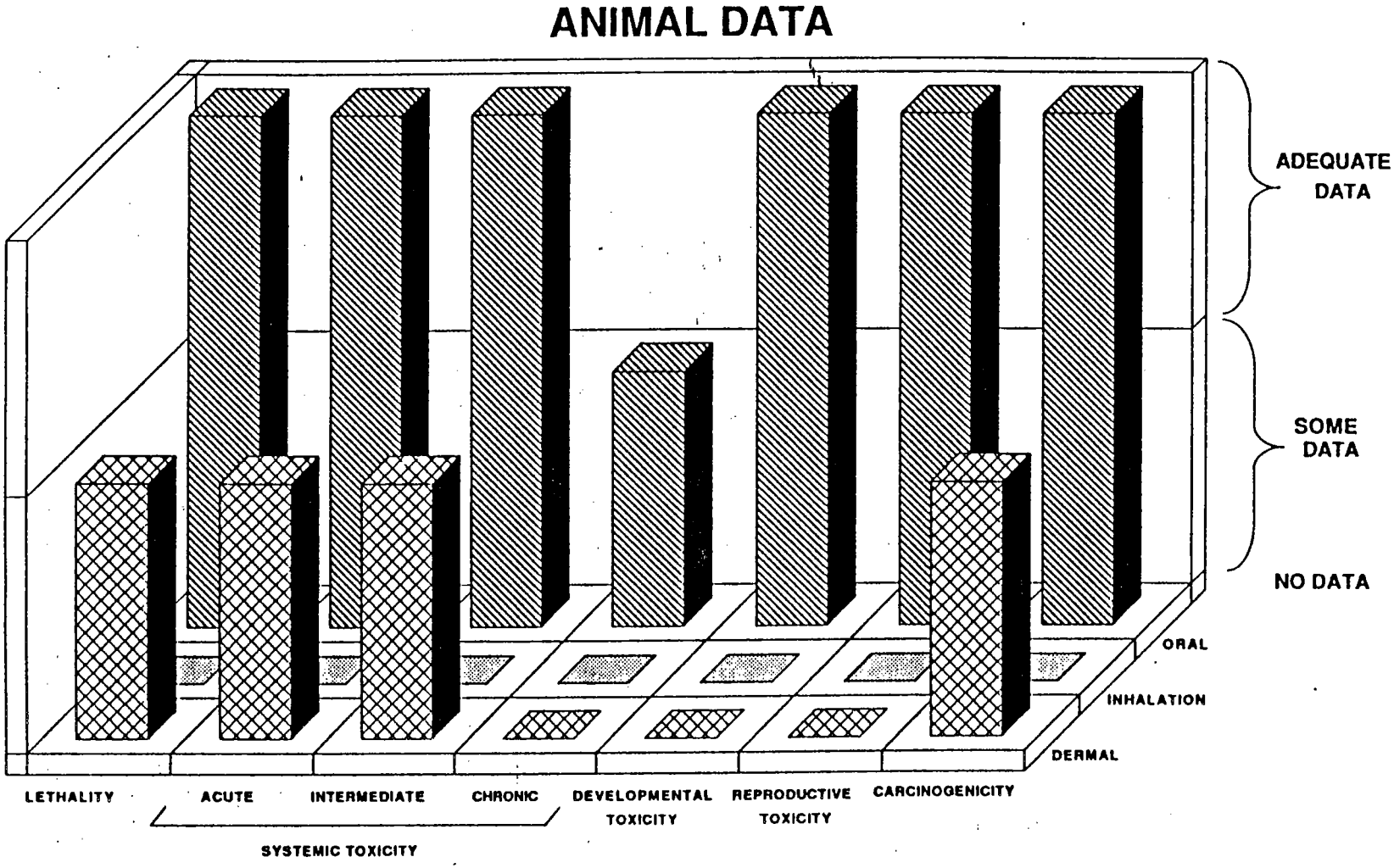


Fig. 2.6. Adequacy of the database on health effects of 2,3,7,8-TCDD (animal data).

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The bars of full height indicate that there are "adequate" data to meet at least one of the following conditions:

1. For noncancer health end points, one or more studies are available that meet current scientific standards and are sufficient to define a range of toxicity from no-effect levels (NOAELs) to levels that cause effects (LOAELs or FELs).

2. For human carcinogenicity, a substance is classified as either a "known human carcinogen" or "probable human carcinogen" by both EPA and International Agency for Research on Cancer (IARC) (qualitative), and the data are sufficient to derive a cancer potency factor (quantitative).

3. For animal carcinogenicity, a substance causes a statistically signficant number of tumors in at least one species, and the data are sufficient to derive a cancer potency factor.

4. There are studies which show that the chemical does not cause this health effect via this exposure route.

Bars of half height indicate that "some" data for the end point exist but do not meet any of the criteria for "adequate" data.

Although adequacy of data is indicated in Fig. 2.5 for dermal exposure only, the route of exposure in the available studies is not clearly defined. Because of the nature of exposures to 2,3,7,8-TCDD, both inhalation and oral exposure are likely to occur along with dermal exposure; in some instances, exposure from these other routes will contribute substantially to the body burden.

2.3.2.2 Descriptions of highlights of graphs

Human. Figure 2.5 indicates that there are very little human data on the toxicological effects of exposure to 2,3,7,8-TCDD. The data obtained from human studies are considered to be from dermal exposures, since most humans were exposed in contaminated areas considerably after the application or initial release of the chemicals containing 2,3,7,8-TCDD. This long-term exposure would occur through contact of the skin with soil and other articles contaminated with 2,3,7,8-TCDD. Inhalation and oral exposures were also likely to occur through inhalation of contaminated dust and ingestion of contaminated food and dust. From reports of human exposure (acute accidental exposure as well as repeated exposure), there is a clear indication that chemicals containing 2,3,7,8-TCDD cause chloracne; there is also some indication that chemicals containing 2,3,7,8-TCDD are hepatotoxic; however, there are no data regarding the levels of exposure required for induction of these effects. The epidemiologic data on the effects of 2,3,7,8-TCDD on fetal development, human reproduction, and the development of cancer are too limited to either prove or disprove an association. As a result of mixed exposure and the lack of quantitative data on populations exposed to a compound in the environment, it is anticipated that filling data gaps on 2,3,7,8-TCDD will be difficult.

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There are no additional data on the toxicity of 2,3,7,8-TCDD following inhalation exposure or oral exposure.

Animal. Figure 2.6 indicates that there are considerably more data on the toxicological effects of 2,3,7,8-TCDD in animals compared to humans, although the data are primarily limited to oral studies. For animal data, it should be recognized that "adequate" may only apply to the specific species tested, since large species differences are observed with 2,3,7,8-TCDD. As a result of these large differences in species sensitivity (4 orders of magnitude for LD_{50} values), complete information on a specific end point will be difficult to obtain. For the complete understanding of a toxicological end point, the most sensitive species must be determined and adequately tested, or it must be clearly demonstrated which animal species is most representative of humans. The lack of adequate data on species sensitivity is a significant gap in the toxicological data on 2,3,7,8-TCDD.

Although dermal exposure is a potentially significant route of human exposure, the only animal data available for this route are a single determination of a lethal dose in rabbits (Schwetz et al. 1973) and a few studies of the effects of 2,3,7,8-TCDD in the two-stage tumorigenesis assay (EPA 1985a) of the mouse. Both of these types of studies provide only limited data on the dermal toxicity of 2,3,7,8-TCDD. This is particularly the case where exposure in humans is likely to occur from 2,3,7,8-TCDD which is adsorbed to soil or other material and may have only limited bioavailability compared with 2,3,7,8-TCDD in dermal studies where the chemical was applied in organic solvents.

2.3.2.3 Summary of relevant ongoing research

A review of *Federal Research in Progress* will show that investigations of the toxicological properties of 2,3,7,8-TCDD are very active. This considerable research effort is too extensive to enumerate individual studies. However, a very important area of ongoing research is reviewed by Young and Kang (1985). This area consists of 15 ongoing epidemiology studies that are being conducted for the U.S. government and coordinated by the White House Agent Orange Working Group. Subject areas include mortality and morbidity, with particular concern for carcinogenic effects, anatomical birth defects, and the development of soft tissue sarcomas. A number of these studies are concerned with veterans exposed to the herbicide Agent Orange in Vietnam.

2.3.3 Adequacy of the Database for Other Information Needed for Risk Assessment

2.3.3.1 Pharmacokinetics and mechanisms of action

Mechanisms of action. The mechanism of 2,3,7,8-TCDD toxicity is under extensive investigation. As reviewed by Roberts et al. (1985), it has been proposed that 2,3,7,8-TCDD functions by a receptor-mediated mechanism. There is evidence that this mechanism is associated with many of the toxicological end points of 2,3,7,8-TCDD, as indicated by the segregation of the toxic properties of 2,3,7,8-TCDD with the Ah locus, which is near the locus for the 2,3,7,8-TCDD receptor. Some toxicologic

end points, however, do not segregate with the Ah locus. It has been shown in tissue cultures with human lymphoblastoid cells that there is a large genetic variation in aryl hydrocarbon hydroxylase inducibility (Nagaya et al. 1985, 1986), and the susceptibility of these cells to the toxicity of 2,3,7,8-TCDD parallels the induction of this monooxygenase system. In addition, the levels of the receptors in a particular species do not necessarily correspond to species sensitivity. Thus, although a receptor-mediated mechanism is well established, the complete integration of this mechanism with the toxic action of 2,3,7,8-TCDD has not been fully characterized.

Rozman et al. (1985) have also described results of a study which indicates that the thyroid hormone thyroxine (T_4) has the ability to modulate the toxicity of 2,3,7,8-TCDD. Male Sprague-Dawley rats were divided into groups of normal rats, thyroidectomized rats, and thyroidectomized rats that received T_4 replacement therapy. Following administration of 2,3,7,8-TCDD, mean time to death and percent mortality at 90 days were similar between the normal rats and the thyroidectomized rats that received T_4 (35 days and 89%, and 37 days and 80%, respectively). Thyroidectomized rats that did not receive T_4 survived longer (mean time to death of 63 days) and had lower mortality at 90 days (44%). It was also demonstrated that the 2,3,7,8-TCDD treatment resulted in a decrease in serum T_4 levels in both normal and thyroidectomized T_4-supplemented rats. The modulation of the lethal effects of 2,3,7,8-TCDD was considered by the authors to be related to thyroid hormone modulation of energy metabolism. The participation of this mechanism in the development of 2,3,7,8-TCDD toxicity needs further study.

In addition, mechanisms for individual end points, such as the wasting syndrome and the development of cleft palate, have also been investigated. Although a number of mechanisms have been proposed for such end points, there has yet to be a definitive understanding of the underlining biochemical processes that result in the observed effects of 2,3,7,8-TCDD. Further investigation of these processes is necessary not only to understand how 2,3,7,8-TCDD induces certain toxic end points, but also how the different target organ responses relate to each other.

Target organ/pharmacokinetic profiles. There are few data to indicate that target organs (such as liver and thyroid) contain relatively higher levels of 2,3,7,8-TCDD than other, nontarget tissues. In general, 2,3,7,8-TCDD appears to distribute to organs in proportion to lipid content (Ryan et al. 1985b) rather than in regard to the sensitivity of the organ to the toxic effect of 2,3,7,8-TCDD. Roberts et al. (1985), however, reported that the distribution of the 2,3,7,8-TCDD receptor may be a better indicator of target organ than the distribution of 2,3,7,8-TCDD itself. Further work is needed to clarify this issue.

Further research is also needed to determine the association between a species' capability to metabolize 2,3,7,8-TCDD and the sensitivity of that species to 2,3,7,8-TCDD-induced toxicity. Although there are data suggesting that the ability to metabolize 2,3,7,8-TCDD is important in determining species sensitivity to this chemical, the data are not complete and a mechanism has not been proven.

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Ongoing research. There are a relatively large number of studies reported in *Federal Research in Progress* that are involved with the toxicokinetics and the mechanism of action of 2,3,7,8-TCDD. The list of projects is too extensive for inclusion in this profile.

2.3.3.2 Monitoring human biological samples

Adequate analytical methods are available to investigate 2,3,7,8-TCDD levels in biological materials that are lipophilic and thus concentrate 2,3,7,8-TCDD. Young (1984) reported that the methodology is not available for detecting 2,3,7,8-TCDD in blood or tissues with low lipid content, although a recent method reported the detection of 2,3,7,8-TCDD in parts-per-quadrillion levels in human serum (Patterson et al. 1987b). The development of more sensitive analytical methods is necessary for environmental analysis, and the resulting technology will be used in monitoring biological samples. At present, the ability to monitor 2,3,7,8-TCDD in human tissues appears to exceed the ability to interpret the toxicological significance of the observed results.

2.3.3.3 Environmental considerations

Analytical methods. Concentrations of 2,3,7,8-TCDD in ambient air and drinking water are so low that the existing analytical methodologies cannot measure the levels in these two media.

Bioavailability. Although some data are available from a few environmental media on the bioavailability of 2,3,7,8-TCDD, a data gap exists on this subject. Bioavailability data are needed, since existing data indicate that 2,3,7,8-TCDD can bind strongly to soils of high organic content, thus decreasing its bioavailability. At present, there are insufficient data on the mechanism of binding to permit adequate estimation of potential absorbed doses from dermal, oral, or inhalation exposure to soils containing 2,3,7,8-TCDD.

Environmental fate and transport. Some progress on the fate and transport of 2,3,7,8-TCDD in environmental media has been made in recent years. Substantial gaps still exist in quantitative data regarding its biodegradability, photolysis, and volatility from environmental media.

Interactions with other common co-contaminants There are no data to indicate that 2,3,7,8-TCDD will interact chemically with other contaminants under environmental conditions. The presence of 2,3,7,8-TCDD in both biological systems and the environment, and the lack of any reactive groups, would support the conclusion that 2,3,7,8-TCDD will not easily react with other compounds.

Ongoing research. Present research efforts are focused primarily on analytical methodology to develop more monitoring data. The objective of most of this research is to develop new analytical methods that will unequivocally identify and quantify very small amounts of 2,3,7,8-TCDD in various environmental media with a faster turnover time. An example of such an ongoing research is the investigation now being performed by Robens and Zabik (n.d.). Substantial research is in progress to determine the concentration of 2,3,7,8-TCDD in serum and human milk samples. Groups of investigators in the Centers for Disease Control, research organizations in Sweden, Rutgers University, the State

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University of New York at Binghamton, and the Canadian Food Research Division are involved in such efforts (Ryan 1987).

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3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

The chemical identity of 2,3,7,8-tetrachlorodibenzo-p-dioxin, to be referred to as 2,3,7,8-TCDD throughout this document, is given in Table 3.1.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

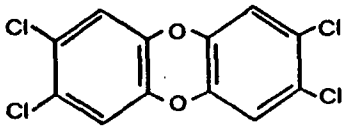
Selected physical and chemical properties of 2,3,7,8-TCDD are shown in Table 3.2. 2,3,7,8-TCDD is stable toward heat, acids, and alkalies, but begins to decompose at 500°C. The decomposition is virtually complete within 21 s at 800°C. 2,3,7,8-TCDD is susceptible to photodegradation in the presence of ultraviolet light, particularly in the presence of a hydrogen-donating solvent. Gamma radiation degrades 2,3,7,8-TCDD in organic solvents (EPA 1984a).

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Table 3.1. Chemical identity of 2,3,7,8-TCDD

Chemical name	2,3,7,8-tetrachlorodibenzo[b,e](1,4)-dioxin; 2,3,7,8-tetrachlorodibenzo-p-dioxin; dioxin; TCDBD; 2,3,7,8-TCDD; 2,3,7,8-tetrachlorodibenzodioxin; 2,3,7,8-tetrachlorodibenzo-1,4-dioxin (EPA 1985)
Trade name	None (The compound is not produced commercially.) (EPA 1985)
Chemical formula	$C_{12}H_4Cl_4O_2$
Wiswesser line notation	TC666 BO IOJ EG FG LG MG or TC666 BO IOJ DG EG LG MG (HSDB 1987)
Chemical structure	
Identification Nos.	
CAS Registry No.	1746-01-6 (SANSS 1987)
NIOSH RTECS No.	HP3500000 (SANSS 1987)
EPA Hazardous Waste No.	Not assigned (HSDB 1987)
OHM-TADS No.	8300192 (SANSS 1984)
DOT/UN/NA/IMCO shipping No.	Not assigned (HSDB 1987
STCC No.	Not assigned (HSDB 1987)
Hazardous Substances Data Bank No.	4151 (HSDB 1987)
National Cancer Institute No.	NCI-CO3714 (SANSS 1987)

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Table 3.2. Physical properties of 2,3,7,8-TCDD

Property	Value	References
Molecular weight	321.97	
Color	Colorless	EPA 1985a
Physical state	Solid at room temperature	EPA 1985a
Odor	Unknown	
Melting point	305°C	Schroy et al. 1985
Boiling point	412.2°C (estimated)	Schroy et al. 1985
Autoignition temperature	NA[a]	
Solubility		
Water (ng/L)	7.91 (20-22°C), 19.3 (22°C), 317 (25°C)	Schroy et al. 1985, Adams and Blaine 1986, Marple et al. 1986a
Organic solvents (mg/L)	o-Dichlorobenzene, 1400; chlorobenzene, 720; benzene, 570; chloroform, 370; methanol, 10; acetone, 110	Schroy et al. 1985
Density (g/mL)	1.827 (estimated)	Schroy et al. 1985
Partition coefficients	Log K_{ow}: 6.15-7.28 Log K_{oc}: 6.0-7.39	EPA 1985a, Schroy et al. 1985, Jackson et al. 1986, Marple et al. 1986b
Vapor pressure (mm Hg)	3.46×10^{-9} (30.1°C), 3.51×10^{-9} (30.2°C), 1.4×10^{-9} (estimated at 25°C)	Rondorf 1986, Schroy et al. 1985, Palansky et al. 1986
Henry's Law constant	2.1×10^{-6} atm-m^3/mol (estimated)	Schroy et al. 1985
Flash point	Unknown	
Refractive index	Unknown	
Flammability limits	Unknown	
Conversion factors		
Vapor	1 ppb = 13.384 $\mu g/m^3$ at 20°C	
Liquid	1 ppb (w/v) = 1 μg/L; 1 ppt (w/v) = 1 ng/L	
Solid	1 ppb (w/w) = 1 μg/kg = 1 ng/g; 1 ppt (w/w) = 1 ng/kg = 1 pg/g	

[a]Not available.

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4. TOXICOLOGICAL DATA

4.1 OVERVIEW

Human and animal data indicate that 2,3,7,8-TCDD can be absorbed following ingestion, and although bioavailability is affected by binding to soils, the extent of absorption is only decreased by ~50%. In addition, animal data indicate that 2,3,7,8-TCDD is absorbed well through the skin. Following absorption, 2,3,7,8-TCDD is distributed to tissues in proportion to the lipid content. 2,3,7,8-TCDD can cross the placenta with subsequent exposure of the fetus, and the newborn can be exposed following redistribution of 2,3,7,8-TCDD during lactation. Metabolism of 2,3,7,8-TCDD is currently thought to result primarily in detoxification and relatively rapid removal of the metabolites through excretion in the bile and urine. Unmetabolized 2,3,7,8-TCDD is also excreted through direct intestinal elimination in feces as well as through lactation. There are species and strain differences in both metabolism and elimination rates, with elimination half-lives varying from 11 days in the hamster, which is relatively resistant to 2,3,7,8-TCDD toxicity, to >1 year in the monkey, which is sensitive to the toxicity of 2,3,7,8-TCDD.

Although humans have been exposed to 2,3,7,8-TCDD as a contaminant of herbicides and industrial chemicals, there have been no reported deaths from acute exposure. Lethal oral doses of 2,3,7,8-TCDD vary from 0.6 to 5000 μg/kg for guinea pigs and hamsters, respectively, with other species tested having LD50 values between these two extremes. Subchronic LD50 values for cumulative (total) exposure during a 90-day oral study with guinea pigs were essentially the same as those observed after acute exposure. A 9-month feeding study of a small number of monkeys indicated that a dose of 2 to 3 μg/kg resulted in death. The acute dermal LD50 value for 2,3,7,8-TCDD in rabbits has been reported to be 275 μg/kg; however, no other species have been tested, and rabbits are only intermediate in sensitivity to 2,3,7,8-TCDD in oral toxicity studies. Inhalation experiments have not been conducted.

The only effect clearly demonstrated to be produced in humans following 2,3,7,8-TCDD exposure is chloracne. This lesion is a systemic toxic effect and not solely a dermal effect. Although animal models are available to study chloracne, there are limitations in investigating this toxicological end point. The lesions produced in animals are different from the lesions in humans, and the expression of this lesion may be limited to monkeys, specific strains of mice, and rabbits (observed only on the ears after dermal application).

In animals, the major toxic effect of 2,3,7,8-TCDD exposure is the wasting syndrome, in which the animals progressively lose body weight prior to death, with no clear signs of altered organ function. Although this syndrome is observed in all species tested, it occurs predominantly

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only at doses that are lethal or near lethal. The wasting syndrome has not been observed in humans.

Immunotoxicity has also been observed in a variety of animal species and may be one of the most sensitive effects of 2,3,7,8-TCDD. Immunotoxicity, however, has not been directly related to 2,3,7,8-TCDD exposure in humans.

Hepatotoxicity has been observed in a variety of animal species, and there is suggestive evidence that this effect also occurs in humans. The human data are not completely clear, however, since mixed exposure to other potentially hepatotoxic chemicals occurred. In addition, species variations in induction of hepatotoxic effects indicate that the two most sensitive species, guinea pigs and monkeys, develop relatively less severe hepatic lesions than many other species.

Other organ systems are also affected by 2,3,7,8-TCDD. There is suggestive evidence in humans that the nervous system may be affected; in animals, there are data on effects on the digestive system and the kidney. These additional organ systems have not been investigated as extensively as those described later. Also, they do not appear to be prime indicators of 2,3,7,8-TCDD toxicity or provide any special insight into extrapolating effects observed in animals to those in man.

Many of the toxic end points discussed have been observed to have extensive species and strain differences in sensitivity. These differences may be related to the receptor-mediated mechanism of toxicity proposed for 2,3,7,8-TCDD. This mechanism follows the scheme of 2,3,7,8-TCDD binding to a soluble cytoplasmic receptor protein, which subsequently migrates into the nucleus of the cell as a receptor-2,3,7,8-TCDD complex. Once in the nucleus, it is proposed that both transcription and translation of DNA may be affected. The receptor protein is known genetically to segregate with the Ah locus, and many of the strain differences in toxicity observed in mice are explained by this segregation. The receptor protein is known to be widely dispersed in different organ systems and in different species, although quantitative levels differ. These quantitative differences may help explain some of the species differences in sensitivity. The receptor mechanism cannot explain all the species and strain differences in 2,3,7,8-TCDD toxicity, because some toxic responses segregate with the Ah receptor while others do not.

The evidence from studies of human populations exposed to herbicides and other industrial chemicals known to be contaminated with 2,3,7,8-TCDD is inadequate to demonstrate that 2,3,7,8-TCDD is a human developmental toxicant. The data from studies in laboratory mice and rats, however, clearly demonstrate in these species that 2,3,7,8-TCDD induces a variety of developmental abnormalities.

The data from human populations exposed to herbicides and other industrial chemicals contaminated with 2,3,7,8-TCDD are not adequate to determine if 2,3,7,8-TCDD adversely affects reproductive health in humans. Animal studies clearly demonstrate that 2,3,7,8-TCDD is fetotoxic in several species and causes spontaneous abortions. There is additional evidence that 2,3,7,8-TCDD affects male hormone levels and the function of male reproductive organs.

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Although 2,3,7,8-TCDD has not consistently produced positive results in genotoxicity assays, there are positive results in diverse bioassay systems which may suggest that 2,3,7,8-TCDD is a genotoxic agent; however, the large numbers of negative results in assays usually predictive of genotoxic agents suggest that 2,3,7,8-TCDD is not a genotoxic agent.

The human evidence that 2,3,7,8-TCDD is a carcinogen is conflicting, with both positive and negative findings reported in cohorts exposed to herbicides and other chlorinated chemicals known to be contaminated with 2,3,7,8-TCDD. As a result, the human data only provide suggestive evidence that 2,3,7,8-TCDD is a human carcinogen. The animal data, however, provide clear evidence that 2,3,7,8-TCDD is carcinogenic in animals.

Exposure to 2,3,7,8-TCDD in the environment is never to 2,3,7,8-TCDD alone but to materials such as incinerator fly ash or industrial wastes, which contain 2,3,7,8-TCDD along with many other congeners of 2,3,7,8-TCDD, as well as other potentially toxic materials. The EPA has recognized the public and toxicological concerns resulting from exposure to these compounds, also the gaps in available information with which to evaluate the human health potential from exposure (EPA 1987). In response to this problem, the Chlorinated dibenzo-p-dioxins/Chlorinated dibenzofurans Technical Panel of the Risk Assessment Forum has developed and recommended an interim method for assisting in estimating the risk from exposure to these chemical mixtures that can be used until the data gaps are filled. This procedure generates the "2,3,7,8-TCDD equivalence factors" (TEFs) of complex mixtures of chlorinated dibenzo-p-dioxins based on congener- and isomer-specific data, and the results are presented in Table 4.1. The TEFs are relative values and are a means of relating toxicity data for a chlorinated dibenzo-p-dioxin to an equivalent level of 2,3,7,8-TCDD. The TEF for 2,3,7,8-TCDD is defined as unity, whereas all other TEFs are unity or less, thus reflecting the lower toxic potency of most 2,3,7,8-TCDD congeners. These relative values were developed using a tiered approach to the evaluation of existing data. The toxicity data were used to extrapolate between congeners and 2,3,7,8-TCDD and were segregated into data types that would provide differing degrees of reliability for the extrapolation process. Using this system, definitive data on the human carcinogenicity of a congener of 2,3,7,8-TCDD would be the most appropriate data for establishing the TEF. If this human data were not available, however, animal carcinogenicity data would be used, followed by the data on reproductive toxicity (estimated exposure levels resulting in reproductive and carcinogenic effects are very similar for 2,3,7,8-TCDD). Finally, if none of the above data are available, the TEFs are determined by the weight of evidence from in vitro tests (with particular weight placed on data from tests evaluating receptor binding interactions and induction of oxidative enzymes). The TEFs thus generated can be used, assuming additivity of the toxic response, for estimating the relative toxicity of a mixture containing a known distribution of congeners of 2,3,7,8-TCDD.

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Table 4.1. Recommended TEFs[a] for 2,3,7,8-TCDD and its congeners

Compound	EPA current recommended values based on various effects
MonoCDDs	0
DiCDDs	0
TriCDDs	0
2,3,7,8-TCDD	1
Other TCDDs	0.01
2,3,7,8-PeCDDs[b]	0.5
Other PeCDDs	0.005
2,3,7,8-HxCDDs[b]	0.04
Other HxCDDs	0.0004
2,3,7,8-HpCDDs[b]	0.001
Other HpCDDs	0.00001
OCDD	0

[a]Toxicity equivalence factors.

[b]Any isomer that contains chlorine in the 2,3,7,8 positions; CDDs = chlorinated dibenzo-p-dioxins.

Source: EPA 1987.

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4.2 TOXICOKINETICS

4.2.1 Absorption

4.2.1.1 Inhalation

Pertinent data regarding the absorption of 2,3,7,8-TCDD by humans or animals following inhalation exposure were not found in the available literature.

4.2.1.2 Oral

Human. The absorption data available are from an elimination study of 2,3,7,8-TCDD in which a male volunteer ingested ^{3}H-2,3,7,8-TCDD in corn oil at a dose of 1.14 ng/kg (Poiger and Schlatter 1986). Measurements of cumulative elimination in the feces and urine, as well as a determination of sequestering in fat via biopsy samples, indicated that >87% of the dose was absorbed. Following absorption, the half-life for elimination was calculated to be 2120 days.

Animal. Studies reviewed in EPA (1985a) show that 2,3,7,8-TCDD is generally well absorbed (50 to 80%) when administered to rats, guinea pigs, or hamsters in a lipophilic vehicle by gavage (Piper et al. 1973, Nolan et al. 1979, Olson et al. 1980). There appeared to be no change in absorption rates with repeated dosing, but there was some decrease in absorption at higher dose levels. Absorption of 2,3,7,8-TCDD when administered in the diet was also between 50 and 60% (Fries and Marrow 1975).

The vehicle in which 2,3,7,8-TCDD is administered has substantial effects on its gastrointestinal absorption. As described in EPA (1985a), Poiger and Schlatter (1980) observed a decrease in the absorption of 2,3,7,8-TCDD when administered in a soil suspension compared with a solution in 50% ethanol, whereas no absorption occurred when the compound was administered as a suspension of activated carbon. Since 2,3,7,8-TCDD in the environment is likely to be adsorbed to soil, McConnell et al. (1984) and Lucier et al. (1986) compared the absorption of 2,3,7,8-TCDD from contaminated soil with that from 2,3,7,8-TCDD administered in corn oil. As indicated by biological effects and the amount of 2,3,7,8-TCDD in the liver, the absorption was ~50% less from soil than from corn oil. Umbreit et al. (1986a) showed that 2,3,7,8-TCDD-contaminated soil was less toxic than an equivalent amount of 2,3,7,8-TCDD, suggesting that binding to soil had an influence on bioavailability. These data indicate that substantial absorption occurs from contaminated soil; however, soil type and duration of contact, as suggested from the data that demonstrated decreased extraction efficiency with increasing contact time between soil and 2,3,7,8-TCDD (Phillipi et al. 1981, Huetter and Phillipi 1982), may substantially affect the absorption of 2,3,7,8-TCDD from soils obtained from different contaminated sites.

4.2.1.3 Dermal

Human. Pertinent data regarding the absorption of 2,3,7,8-TCDD following dermal exposure in humans were not found in the available literature.

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Animal. Data regarding dermal absorption in animals are limited. 2,3,7,8-TCDD is absorbed well through the skin, although as with oral absorption the vehicle can substantially affect absorption. As discussed in EPA (1985a), it was estimated that 40% of the dose was absorbed by rats when the compound was applied in methanol, whereas application in vaseline or polyethylene glycol resulted in 1.4 and 9.3% absorption, respectively (Poiger and Schlatter 1980). Applying 2,3,7,8-TCDD as a paste in soil or activated carbon resulted in poorer absorption than observed in oral studies, with absorption of <2% and nondetectable, respectively.

4.2.2 Distribution

4.2.2.1 Inhalation

Pertinent data regarding the distribution of 2,3,7,8-TCDD following inhalation exposure of humans and animals were not found in the available literature.

4.2.2.2 Oral

Human. Poiger and Schlatter (1986) estimated that ~90% of the body burden of 2,3,7,8-TCDD was sequestered in the fat after a volunteer ingested ^{3}H-2,3,7,8-TCDD in corn oil at a dose of 1.14 ng/kg. During this study, which lasted 135 days, elevated radioactivity was detected in the blood only during the initial 2 days following treatment. The data would be consistent with the high bioconcentration potential of 2,3,7,8-TCDD in humans, as calculated by Geyer et al. (1986) from daily intake assumptions, levels in human adipose tissue, and pharmacokinetic models. Adipose tissue has been examined to determine if levels of 2,3,7,8-TCDD in this reservoir correlated with exposure. Adipose tissues of Vietnam veterans exposed to Agent Orange and humans occupationally exposed to potential sources of 2,3,7,8-TCDD were reported to have up to 10 times the level of 2,3,7,8-TCDD in unexposed control subjects (Schecter et al. 1985, Gross et al. 1984); however, another study by Weerasinghe et al. (1986) failed to detect any difference between a group of veterans and unexposed subjects. Although a clear correlation with exposure was not demonstrated, it was apparent that adipose tissue is a depot for 2,3,7,8-TCDD, and detectable levels were observed in subjects with no known exposure. Ryan et al. (1985b) further examined the distribution of 2,3,7,8-TCDD in two humans at autopsy. They determined on a weight basis that 2,3,7,8-TCDD distributed in descending order to fat (~6 ppt) and liver (~2 ppt), with levels in muscle and kidney below detection; however, 2,3,7,8-TCDD levels compared on a per lipid basis were similar between tissues. These data should be interpreted with caution, since only two subjects were examined and one of the subjects was suffering from fatty liver syndrome; therefore, the data cannot be generalized to the entire population.

Animal. EPA (1985a) reviewed a number of early studies that described the distribution of 2,3,7,8-TCDD in rats, mice, and guinea pigs. In rats and mice, the liver contained the greatest amount of 2,3,7,8-TCDD, followed by the fat; however, in guinea pigs this was reversed. Similar patterns were observed after intraperitoneal administration. In studies of congenic mice which differ only at the Ah

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locus, the distribution patterns were similar to those previously described, except that relatively higher levels of 2,3,7,8-TCDD were reported in the livers of the responsive mice compared with the nonresponsive strain (Gasiewicz et al. 1983a,b; Birnbaum 1986). Thus, it appears that there is little potential for strain difference in the distribution pattern of 2,3,7,8-TCDD. Lakshmanan et al. (1986) reported that the tissue distribution of 2,3,7,8-TCDD is mediated through transport in the lymph system in the rat, with initial high levels of 2,3,7,8-TCDD in the lymph decreasing rapidly with progressive accumulation in the fat during the first 24 h. The half-life for redistribution of 2,3,7,8-TCDD out of adipose tissue and liver was 7.6 and 5.3 weeks, respectively.

2,3,7,8-TCDD crosses the placenta and accumulates in the mouse fetus after gavage administration (Weber and Birnbaum 1985). In this study, ~0.5% of the dose was detected in the fetus. Similar results were reported by Krowke (1986) in mice treated with 2,3,7,8-TCDD by subcutaneous injection. Fetal distribution was not uniform, with 75% of the 2,3,7,8-TCDD located in the fetal liver. In addition to in utero exposure of the fetuses, Nau et al. (1986) reported that postnatal exposure of the young occurred via the milk after a single administration of 2,3,7,8-TCDD to pregnant mice between gestation days 14 and 17. Again, the fetal and pup liver was the predominant storage site.

4.2.2.3 Dermal

Human. Studies of humans exposed to herbicides or other industrial compounds known to be contaminated with 2,3,7,8-TCDD were discussed in Sect. 4.2.2.2 (oral distribution) for easier comparison with the only available human experimental data. Routes of exposure in these studies are not clearly defined; the most likely route is probably dermal, although oral and inhalation exposure are also likely to occur.

Animal. No studies are available.

4.2.3 Metabolism

Pertinent data regarding the metabolism of 2,3,7,8-TCDD following inhalation or dermal exposure of humans or animals, or following oral exposure of humans, were not found in the available literature. However, data regarding the metabolism of 2,3,7,8-TCDD following oral exposure of animals are available.

2,3,7,8-TCDD is relatively slow to metabolize, but once metabolites are formed they are rapidly excreted through the urine and bile as glucuronide and sulfate conjugates (Olson et al. 1980, 1983, as reviewed in EPA 1985a). Recently, however, Olson (1986) reported that up to 28% of the radioactivity in the tissues of guinea pigs treated with labeled 2,3,7,8-TCDD was associated with metabolites, indicating that under the conditions used, the metabolites of 2,3,7,8-TCDD were not eliminated as rapidly as previously believed. Sawahata et al. (1982) identified 1-hydroxy-2,3,7,8-tetrachlorodibenzo-p-dioxin and 8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin in vitro, and Poiger et al. (1982) identified 2-hydroxy-1,3,7,8-tetrachlorodibenzo-p-dioxin in dogs as major metabolites of 2,3,7,8-TCDD. Other hydroxylated products were identified,

as minor metabolites. Mason and Safe (1986a,b) synthesized some of these metabolites and demonstrated that they had considerably less biological activity than 2,3,7,8-TCDD, which supports the observation by Weber et al. (1982) and Poiger et al. (1982) that metabolites extracted from the bile of dogs treated with 2,3,7,8-TCDD were less toxic to rats and guinea pigs, respectively, than equivalent amounts of 2,3,7,8-TCDD.

Species and strain differences in the metabolism of 2,3,7,8-TCDD have been investigated to determine if differences in metabolism could account for the large observed difference in toxicity. Gasiewicz et al. (1983b) reported that qualitative evaluations of the elution profiles of the urinary and biliary metabolites of 2,3,7,8-TCDD were similar for responsive and nonresponsive strains of mice. In dogs (in vivo) and rats (in vitro), pretreatment with 2,3,7,8-TCDD resulted in a 100 and 320% increase, respectively, in the rate of metabolism of a subsequent dose of 2,3,7,8-TCDD, whereas in guinea pigs (in vitro), pretreatment with 2,3,7,8-TCDD had no effect on the rate of metabolism of a subsequent dose of 2,3,7,8-TCDD (Poiger and Schlatter 1985, Wroblewski and Olson 1985, Olson and Wroblewski 1985). These data suggest that some of the differences in the toxicity of 2,3,7,8-TCDD may be related to the rate of metabolism as well as qualitative and quantitative differences in the metabolites formed.

4.2.4 Excretion

Pertinent data regarding the excretion of 2,3,7,8-TCDD following inhalation or dermal exposure of humans or animals were not found in the available literature. However, data regarding the excretion of 2,3,7,8-TCDD following oral exposure of humans and animals are available.

4.2.4.1 Human

Poiger and Schlatter (1986) studied the elimination profile of 2,3,7,8-TCDD from a volunteer who ingested a single 1.14-ng/kg dose of the tritiated chemical. Urinary levels of radioactivity were never above background levels during the 135 days the individual was studied. Fecal elimination was initially rapid, with 11% of the dose eliminated in the first 3 days (speculated to be nonabsorbed material), whereas during days 7 to 125, only 3.5% of the dose was eliminated. From these data, a half-life of 2120 days in the body was calculated assuming first-order kinetics. It is, however, not known with any certainty that 2,3,7,8-TCDD is actually eliminated by first-order kinetics, and other kinetic parameters would change the estimated half-life.

4.2.4.2 Animal

2,3,7,8-TCDD and its metabolites are eliminated predominantly in the feces and in the urine in an apparent first-order process. There appear to be no major differences whether the material was administered by the oral or intraperitoneal routes (EPA 1985a). Although metabolites were observed in the bile, unmetabolized 2,3,7,8-TCDD detected in the feces probably was derived from direct intestinal elimination, as demonstrated by Olson (1986) in guinea pigs. Nau et al. (1986) reported that elimination of 2,3,7,8-TCDD through lactation is significant in mice. EPA (1985a) reported that between 91 and 99% of the excreted

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2,3,7,8-TCDD-derived radioactivity was found in the feces of rats and guinea pigs; 54 and 72% was detected in the feces of mice, with greater fecal excretion in the responsive C57Bl/6J strain; and 59% was observed in the feces of hamsters. Half-lives for elimination also varied with species and strain as follows (in decreasing order): guinea pigs (22 to 30 days); rats (17 to 31 days); mice (11 to 24 days, 11 days in responsive mice and 24 days in nonresponsive strains); and hamsters (10 to 15 days). Olson (1986) reported even longer half-lives of >90 days in guinea pigs. Although there are no adequate excretion data from monkeys, there are indications that 2,3,7,8-TCDD is very persistent in this species, with a possible half-life of >1 year (EPA 1985a).

4.3 TOXICITY

4.3.1 Lethality and Decreased Longevity

4.3.1.1 Inhalation

Pertinent data regarding lethality and decreased longevity following inhalation exposure of humans or animals to 2,3,7,8-TCDD were not found in the available literature.

4.3.1.2 Oral

Human. No studies are available.

Animal. Results of extensive investigations of the acute lethality of 2,3,7,8-TCDD indicate that this chemical is toxic at very low levels in all species tested. Species differences in lethality cover 4 orders of magnitude in dose level. LD_{50} values, after gavage administration in lipophilic solvents, were reported to range from 0.6 to 2.1 μg/kg in Hartley guinea pigs (Schwetz et al. 1973), 20 to 60 μg/kg in rats, 100 to 600 μg/kg in mice, and 1000 to 5000 μg/kg in hamsters (EPA 1985a, McConnell 1985). Rhesus monkeys have also been studied, but the lowest dose tested, 70 μg/kg, was lethal (McConnell et al. 1978). Reproductive toxicity studies indicate that female rhesus monkeys are very sensitive to the lethal effects of 2,3,7,8-TCDD. Eight of 16 pregnant monkeys died after treatment with a total dose of 1.0 μg/kg over a 9-day period during gestation (McNulty 1985); toxic effects, as indicated by abortion, were observed at a total 2,3,7,8-TCDD dose of 0.2 μg/kg. Following acute administration, death is generally observed only after an extended period of time that ranges from 5 to 40 days; this lag time appears to be, to some extent, independent of dose after a threshold is reached.

Other factors besides species differences affect the acute toxicity of 2,3,7,8-TCDD. These variables include the strain of animal tested, with responsive C57BL/6J mice demonstrated to be twice as sensitive to 2,3,7,8-TCDD-induced lethality as DBA/2J nonresponsive strains (Gasiewicz et al. 1983a,b), and differences in lethal dose (ranging from 165 to 320 μg/kg) reported for four strains of rats (Walden and Schiller (1985). Strain differences have not been investigated in other species but would be anticipated to occur. Also, the vehicle substantially affects the acute lethality of 2,3,7,8-TCDD, probably by altering the bioavailability of the compound. However, as discussed by Kaminsky et

al. (1985) and illustrated by Umbreit el al. (1985, 1986a,b), the influence of matrix on bioavailability is complex, with factors such as the properties of the matrix as well as duration of contact with the matrix substantially altering the bioavailability. The complexity of this issue is further illustrated by the protective effect of orally administered activated charcoal on the lethality of subcutaneously or intraperitoneally administered 2,3,7,8-TCDD (Manara et al. 1984), suggesting that some of the effects are the result of processes other than bioavailability.

The lethal effects of subchronic exposure to 2,3,7,8-TCDD have been studied by DeCaprio et al. (1986) in Hartley guinea pigs. Guinea pigs were maintained on diets containing 2, 10, 76, or 430 ppt of 2,3,7,8-TCDD for 90 days. At 76 and 430 ppt, mortalities of 10 and 70%, respectively, were observed, and an LD_{50} value of 0.8 μg/kg for the total consumption of 2,3,7,8-TCDD was calculated. This value is similar to that observed in the previously described study of the acute exposure of male guinea pigs to 2,3,7,8-TCDD. Exposures of 0.61 ng/kg/day resulted in no deaths. In rhesus monkeys, however, Allen et al. (1977) observed deaths in 5 of 8 animals maintained for 9 months on a diet that contained 500 ppt of 2,3,7,8-TCDD. From food intake, the total dose ingested was calculated to be 2 to 3 μg/kg or 0.0093 to 0.014 μg/kg/day.

4.3.1.3 Dermal

Human. No studies are available.

Animal. There is only one study available regarding the lethality of 2,3,7,8-TCDD by the dermal route. Schwetz et al. (1973) determined that the acute LD_{50} of 2,3,7,8-TCDD in acetone was 275 μg/kg (range, 142 to 531 μg/kg) in New Zealand white rabbits. As was observed after oral administration, the time to death was protracted, with deaths observed between 12 and 22 days after treatment.

4.3.2 Systemic/Target Organ Toxicity

4.3.2.1 Chloracne

Inhalation. Pertinent studies regarding the development of chloracne by humans or animals following inhalation exposure were not found in the available literature.

Oral, human. No studies are available.

Animal. Greig (1984) observed typical skin lesions in male and female hairless A2G-hr/+ mice 4 weeks after a single gavage administration of 2,3,7,8-TCDD at 75 μg/kg. The females appeared to be more severely affected than the males. McConnell et al. (1978) observed acneform eruptions in rhesus monkeys given a single dose of 2,3,7,8-TCDD at 70 μg/kg. This dose was severely toxic, resulting in many gross effects and ultimately death. Longer-term exposure of monkeys to 2,3,7,8-TCDD in the diet at 500 ppt (0.0094 to 0.014 μg/kg/day) for 9 months also produced lesions that resembled chloracne (Allen et al. 1977). This dose was not a threshold for the formation of chloracne, since the lesions were severe and associated with other dermal effects such as subcutaneous edema and loss of facial hair. In addition, this

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level of 2,3,7,8-TCDD resulted in the deaths of 5 of 8 animals tested.

Dermal, human. Since the turn of the century, chloracne has been observed in humans after a few days from the time of accidental exposure to a variety of chlorinated aromatic compounds. The prevalence of these lesions has been reviewed by Taylor (1979) and Suskind (1985). 2,3,7,8-TCDD is known to be one of the most potent compounds in producing chloracne; however, sufficient data on exposure are not available to define the doses necessary to produce this lesion. It is known that chloracne appears prior to any other visible effects related to 2,3,7,8-TCDD exposure. The lesions usually appear on the face and upper trunk area. These lesions can be very disfiguring and can be persistent, lasting many years after exposure. After the Seveso accident, children appeared to develop chloracne more frequently than adults, but this may have been related to greater exposure to contaminated soils during play rather than to a greater sensitivity in the young.

Additional signs of toxicity have been reported in case studies of small groups of people exposed to 2,3,7,8-TCDD. These signs include aching muscles, loss of appetite, weight loss, digestive disorders, easy fatigability, insomnia, loss of libido, headache, neuropathy, sleep disturbance, sensory changes, and uncharacteristic bouts of anger (Poland et al. 1971, Bauer et al. 1961, Kimmig and Schulz 1957, Schulz 1957, Oliver 1975). Many of these symptoms are commonly observed with acute exposure to chemicals, and since the exposure to 2,3,7,8-TCDD is always a mixed exposure, with 2,3,7,8-TCDD being only a minor component (on a percentage basis), it is difficult to state with certainty that these symptoms are produced from 2,3,7,8-TCDD. The other chemicals that provide concomitant exposure not only include the chlorinated compound of which 2,3,7,8-TCDD is a contaminant, but, in many cases, such as with herbicide use, petroleum solvents as well as other "inert ingredients" used in the formulation of the final product.

Dermal, animal. Puhvel et al. (1982) applied 0.1 μg of 2,3,7,8-TCDD to the skin of hairless Skh:HR-1 mice 3 times/week for 4 weeks. The mice developed skin lesions which appeared on histologic examination to resemble chloracne. Changes in the skin included hyperkeratinization, hyperplasia, absences of sebaceous glands, and buildup of keratin into dermal cysts. 2,3,7,8-TCDD was the most effective agent of the seven tested in inducing this dermal response, with the next most effective compound, 3,4,3',4'-tetrachlorobiphenyl, inducing a response at 200 μg per application. The use of a single dose in this study precludes the determination of a threshold dose.

Toth et al. (1979) administered 2,3,7,8-TCDD to Swiss mice by gavage for 1 year at doses of 0, 0.007, 0.7, or 7.0 μg/kg/week. Amyloidosis was observed in the kidney, spleen, and liver, along with dermatitis, in all treatment groups. The dermatitis had some similarities to chloracne, although it is not clear if the etiology is the same. If this dermatitis is similar to chloracne, then the LOAEL in mice would be 0.007 μg/kg/week.

General discussion. Chloracne is a persistent deformative skin lesion that can be induced by single or multiple exposure to 2,3,7,8-TCDD in humans. Chloracne is the first toxic effect usually observed in humans exposed to chemicals contaminated with 2,3,7,8-TCDD and appears

to be a sensitive toxicological end point. It is believed that humans can develop chloracne following exposure to 2,3,7,8-TCDD by any route, but data are not available regarding the dose necessary to induce chloracne in humans.

Moses and Prioleau (1985) studied humans who had recovered from chloracne and determined that histologic examination of the skin was incapable of providing any indication of past exposure to 2,3,7,8-TCDD. Only a few experimental animals develop chloracne upon exposure to compounds that produce this lesion in humans; the limitation of appropriate animal models has resulted in a poor understanding of this lesion and little information on dose-response relationships. In vitro studies by Greenlee et al. (1984) using human epidermal cultures suggested that 2,3,7,8-TCDD may affect the receptors for epidermal growth factors, which ultimately results in the typical dermal lesions associated with 2,3,7,8-TCDD exposure.

4.3.2.2 Wasting syndrome

Inhalation. Pertinent data regarding wasting syndrome in humans or animals following inhalation exposure to 2,3,7,8-TCDD were not found in the available literature.

Oral, human. No studies are available.

Animal. The wasting syndrome has been observed in several species after administration of acute lethal doses of 2,3,7,8-TCDD (EPA 1985a). As described in the above review, body weight will decrease after administration of 2,3,7,8-TCDD, possibly in a biphasic pattern, until death occurs 15 to 30 days after exposure. Studies with pair-fed rats suggest that the wasting syndrome results from 2,3,7,8-TCDD-induced appetite suppression rather than malabsorption or altered food energy utilization (Rozman 1984; Seefeld and Peterson 1984; Seefeld et al. 1984a,b; Kellin et al. 1985; Potter et al. 1986). Recently, however, Lu et al. (1986) demonstrated that total parenteral nutrition only protected Hartley guinea pigs partially from loss of weight. Treated guinea pigs maintained relatively stable body weight until a few days prior to death, demonstrating that decreased food consumption accounts for most of the observed loss in body weight but that other mechanisms must account for the final decrease in weight and ultimate death. Additional studies in cold-adapted Sprague-Dawley rats, which maintain high levels of food intake after treatment with 2,3,7,8-TCDD, support the observation that decreased food consumption only partially accounts for the wasting syndrome, since the cold-adapted rats lost weight twice as fast as rats treated at normal temperatures (Rozman and Greim 1986).

Rhesus monkeys and Hartley guinea pigs also lose body weight after prolonged exposure to diets containing 2,3,7,8-TCDD (Allen et al. 1977, DeCaprio et al. 1986). Weight loss was ~20% in female monkeys ingesting 2,3,7,8-TCDD at ~0.01 μg/kg/day (the only dose level studied) for 9 months. Only eight monkeys were tested and five died at this dose. Guinea pigs (10 males and 10 females) maintained on diets for 90 days which provided doses of 2,3,7,8-TCDD of 0.0049 μg/kg/day had a 15% decrease in body weight, whereas guinea pigs given doses of 0.026 μg/kg/day had a 40% decrease in body weight. All guinea pigs in the high-dose group died or were killed (when moribund) before the end of

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the study. The 0.00061-μg/kg/day level was a no-effect level. It appears that even in long-term studies, the severe loss of body weight, characteristic of exposure to 2,3,7,8-TCDD, is associated with dose levels that are lethal to the animal.

Dermal. Pertinent data regarding the wasting syndrome in humans or animals exposed dermally to 2,3,7,8-TCDD were not found in the available literature.

General discussion. The wasting syndrome is a characteristic sign of 2,3,7,8-TCDD toxicity in experimental animals. This syndrome is observed in both acute and longer-term studies and is most commonly associated with lethal doses. The wasting syndrome has not been observed in humans.

Since the mechanism is not clearly understood, it remains to be determined whether the severe body weight loss associated with 2,3,7,8-TCDD toxicity is the cause of the subsequent death of the animal or an effect that can be segregated from lethality. Mechanisms have been proposed by Aust (1984) which suggest that the effect of 2,3,7,8-TCDD on the thyroid results in activation of thyrotropin-releasing hormone, which has an anorectic action, and, in conjunction with 2,3,7,8-TCDD-induced vitamin A depletion, results in loss of body weight. Regardless of the mechanism or length of exposure, the wasting syndrome appears to be an indicator of impending death rather than an early sign of toxicity. No reports of abnormal weight change as a result of 2,3,7,8-TCDD exposure in humans were found.

4.3.2.3 Hepatic effects

Inhalation. Pertinent data regarding hepatotoxicity in humans or animals exposed by inhalation to 2,3,7,8-TCDD were not found in the available literature.

Oral, human. No studies are available.

Animal. The acute administration of 2,3,7,8-TCDD to rats and mice results in toxic effects in the liver, whereas no severe changes in the liver are observed upon acute administration of 2,3,7,8-TCDD to guinea pigs and monkeys (EPA 1985a). Lesions in rodents consist of necrosis, proliferative changes, cellular membrane alterations, bile duct proliferation, altered lipid metabolism, and excess amounts of porphyrin. These liver effects have generally been observed following a single exposure at relatively high doses of 5 to 200 μg/kg.

Turner and Collins (1983), however, did observe morphologic changes in a small number of guinea pigs (groups of 1 male and 4 to 6 females, strain not reported) given a single gavage dose of 2,3,7,8-TCDD at 0.1, 0.5, 2.5, 12.5, or 20 μg/kg. Changes including hypertrophy, steatosis, focal necrosis, and hyalin-like bodies were reported in all guinea pigs, although no indication of an association between dose and increased severity or incidence was reported. Deaths were reported for doses >0.5 and 12.5 μg/kg in males and females, respectively. In a 90-day feeding study with Hartley guinea pigs, similar mild liver changes were observed in animals maintained on diets that provided doses of 0.0049 μg/kg/day; liver changes were not observed at the next lower dose of 0.00061 μg/kg/day (DeCaprio et al. 1986). Although chronic studies are not

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available in guinea pigs, studies reviewed by EPA (1985a) in rats (Kociba et al. 1979, NTP 1982a) and mice (NTP 1982a) indicated that doses that represent a LOAEL of ~0.001 μg/kg/day for 2 years are also hepatotoxic.

Dermal, human. Studies of the hepatotoxic effects of 2,3,7,8-TCDD on humans have been conducted on populations exposed to herbicides and other industrial chemicals contaminated with 2,3,7,8-TCDD. In these studies, it is considered that the predominant route of exposure is dermal, although some exposure by the oral and inhalation routes would also be expected. In addition, all individuals studied were exposed to multiple chemicals, which may have contributed to or been the cause of the effects observed.

Reports reviewed by EPA (1985a), May (1973), Holden (1979), Bogen (1979), and Holmstedt (1980) have reported "liver dysfunction" as one of the symptoms in workers and Vietnam veterans exposed to the herbicide 2,4,5-T contaminated with 2,3,7,8-TCDD. Since these were essentially case reports, no firm association between exposure and effect could be made. A more complete clinical study of the levels of serum liver enzyme was conducted in children ages 6 to 10 who were potentially exposed to 2,3,7,8-TCDD in the Seveso accident (Mocarelli et al. 1986). The children were examined yearly for 6 years following the accident. During the initial 2 years, elevated serum levels of gamma-glutamyltransferase (GGT) and alanine aminotransferase (ALT) were observed in both boys and girls who resided in the most highly contaminated area. The increase was slight, with a total incidence of 4.3% compared to 3.6% in the control group. Values returned to normal after 2 years. Ideo et al. (1982) reported elevated urinary D-glucaric acid in the urine of children in the Seveso area ~2.5 years after the accident. They also noted that there was a strong correlation between glucaric acid levels and the activity of hepatic microsomal enzymes. No liver effects, as indicated by serum enzyme levels, however, were observed by Falk et al. (1984) in a pilot epidemiologic study of 122 persons exposed in Missouri to chemicals contaminated with 2,3,7,8-TCDD.

Liver involvement, as indicated by porphyria cutanea tarda, has also been reported to be associated with 2,3,7,8-TCDD exposure. The major studies have been reviewed by Jones and Chelsky (1986), who reported that in all cases the porphyria cutanea tarda may not have been definitively diagnosed or that the other chemicals to which subjects were exposed may have been the causative agents. The pilot epidemiology study by Falk et al. (1984) of the Missouri accident failed to detect porphyria cutanea tarda in a population with known exposure.

Dermal, animal. No studies are available.

General discussion. Hepatotoxic effects have been investigated in human populations exposed to chemicals contaminated with 2,3,7,8-TCDD because of the known hepatotoxicity of 2,3,7,8-TCDD in rodents. Results of these studies have generally been inconsistent with regard to the detection of altered liver serum enzymes or porphyria cutanea tarda. These inconsistencies may be related to the populations studied, the level of contamination, or the bioavailability of the 2,3,7,8-TCDD under the specific conditions of the contaminated site. These confounding factors, along with the ubiquitous nature of and causative agents for

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slight changes in liver function, have made confirmation of 2,3,7,8-TCDD-induced adverse effects on the liver in humans impossible. In rats and mice, 2,3,7,8-TCDD clearly produces adverse effects on the liver; however, there are no studies available that investigated the dose-response relationship; thus, it is not known if initial changes in the liver are a first toxic manifestation of 2,3,7,8-TCDD exposure or an effect observed only after other toxic effects in other organ systems have been manifested.

Although Turner and Collins (1983) reported mild liver effects in guinea pigs (a species generally resistant to 2,3,7,8-TCDD-induced liver damage) at doses as low as 0.1 μg/kg, the lack of association between increasing dose and increasing severity of effect in this study makes it difficult to determine a threshold dose. Observing similar liver changes in a subchronic study in guinea pigs and in chronic studies in rats and mice, however, supports the observation of the acute study that hepatotoxicity is a sensitive indicator of 2,3,7,8-TCDD toxicity.

Additionally, induction of hepatic microsomal enzymes has often been associated with exposure of laboratory animals to 2,3,7,8-TCDD. Early studies by Buu-Hoi et al. (1972) suggested that the toxicity of 2,3,7,8-TCDD was related to altered enzyme function. Later studies, however, have demonstrated that 2,3,7,8-TCDD toxicity appears in many cases to segregate with induction of enzyme activity rather than be the cause of the toxic response. This has been demonstrated for DT-diaphorase (Beatty and Neal 1976) and for mixed function oxidase (Poland et al. 1979, Greig 1979, Kociba and Schwetz 1982, Poland and Knutson 1982). In addition, the toxicity of structurally related chlorinated dibenzo-p-dioxins correlates well with the relative ability of these compounds to induce enzyme activity (Poland and Glover 1973).

4.3.2.4 Immunotoxicity

Inhalation. Pertinent data regarding immunotoxic effects of 2,3,7,8-TCDD following inhalation exposure of humans or animals were not found in the available literature.

Oral, human. No studies are available.

Oral, animal. 2,3,7,8-TCDD has been extensively investigated for immunotoxicity in mice, rats, and guinea pigs, and as indicated in the review by EPA (1985a) and Knutsen (1984), this is a sensitive end point of toxicity. Most studies were conducted with weekly exposures for durations of between 4 and 8 weeks, and minimum effective doses ranged from 1 μg/kg/week for mice to 5 μg/kg/week for rats, and 0.04 μg/kg/week guinea pigs. As the study by Vos et al. (1973) indicates, the Hartley guinea pig may be the most sensitive species, with a LOAEL of 0.04 μg/kg/day and a NOAEL of 0.008 μg/kg/week. In addition to species differences, strain differences in sensitivity to the immunotoxic effects of 2,3,7,8-TCDD have been observed in mice, with immunotoxic sensitivity segregating with the Ah locus. Segregation of immunotoxicity has also been demonstrated in fetal thymus organ cultures in vitro (Dencker et al. 1985). Thymus cultures from C57Bl/6 mice, which are Ah responsive, were very sensitive to the toxicity of 2,3,7,8-TCDD (EC_{50} of 10^{-10} *M*) compared with no observed effects on thymus cultures from the nonresponsive DBA/2J mouse at 3×10^{-8} *M*, the highest concentration

tested. Effects of 2,3,7,8-TCDD on the immune system included decreases in thymus weight, sensitization to antigens (bacterial antigens, skin grafts, and tumor cell development), serum immunoglobins, and B but not T lymphocytes. Thigpen et al. (1975) demonstrated that C57BL/6JFh mice that received as little as 1 μg/kg of 2,3,7,8-TCDD once a week for 4 weeks were more susceptible to death from subsequent bacterial infection. This dose of 2,3,7,8-TCDD did not result in any gross signs of toxicity, suggesting that host susceptibility was an early effect of 2,3,7,8-TCDD exposure. Immunotoxic effects have also been reported in pups of Fischer rats and B6C3F1 mice following in utero exposure and postnatal exposure through lactation (Luster et al. 1982). The effective doses on a maternal weight basis were approximately the same as those that produced effects in adults. The study by Greenlee et al. (1985), using thymic epithelium cell cultures, provides evidence that 2,3,7,8-TCDD acts directly on the epithelium cells by inhibiting the maturation of the thymocytes. Longer-term studies of the effect of 2,3,7,8-TCDD on the immune system are not available.

Dermal, human. There is little information on the immunotoxic effects of 2,3,7,8-TCDD in humans exposed to herbicides or other chemicals contaminated with 2,3,7,8-TCDD. In a pilot epidemiologic study of 82 high-risk and 40 low-risk subjects from areas in Missouri where 2,3,7,8-TCDD exposure occurred, Stehr et al. (1986) failed to detect any signs of immunotoxicity. In a study of 154 exposed and 155 unexposed persons in Gray Summit, Missouri, Hoffman et al. (1986) reported that the exposed group had an increased frequency of anergy (11.8% vs 1.1%) and relative anergy (35.3% vs 11.8%). The exposed group also had non-statistically significant increased frequencies of abnormal T-cell subset test results (10.4% vs 6.8%), a T4/T8 ratio of <1.0 (8.1% vs 6.4%), and an abnormality in the functional T-cell test results (12.6% vs 8.5%). Although the effects have not resulted in an excess of clinical illness in the exposed group, these data suggest that exposure to 2,3,7,8-TCDD may be associated with depressed cell-mediated immunity; however, corroborative studies will be carried out.

Dermal, animal. No studies are available.

General discussion. There are abundant animal data that indicate that immunotoxicity may be one of the most sensitive toxicologic end points for 2,3,7,8-TCDD. There have been very limited investigations of this end point in humans; thus, the importance of 2,3,7,8-TCDD-induced immunotoxicity in humans cannot be evaluated. The time of onset, the duration of the altered immune response, and the fact that evaluation of altered immune response is a specialized analysis not routinely performed in clinical settings will make the assessment of this end point in humans difficult.

The animal data show that 2,3,7,8-TCDD produces immunologic effects in a number of species, and most of the studies have been concerned with the investigation of alterations induced in the immune system. There are less data available regarding dose-response relationships and species and strain differences in sensitivity; these data would be of assistance in evaluating response with regard to human health. Also, no data are

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available on the immunotoxicity of 2,3,7,8-TCDD in monkeys, a species that appears to be very sensitive to other toxic effects of 2,3,7,8-TCDD.

4.3.3 Developmental Toxicity

4.3.3.1 Inhalation

Pertinent data regarding the developmental toxicity of 2,3,7,8-TCDD following inhalation exposure of humans or animals were not found in the available literature.

4.3.3.2 Oral

Human. No studies are available.

Animal. 2,3,7,8-TCDD has been extensively studied for developmental toxicity, and these studies, reviewed in EPA (1985a), indicate that 2,3,7,8-TCDD is teratogenic in mice and rats after gavage administration. In mice, the most commonly observed developmental effects were hydronephrotic kidney and cleft palate (Moore et al. 1973, Neubert and Dillmann 1972, Smith et al. 1976, Courtney 1976). Both of these anomalies have been observed at doses as low as 1 μg/kg (Courtney, 1976, Smith et al. 1976), with the kidney effects observed after only a single exposure on day 10 of gestation. In rats, gavage administration of 2,3,7,8-TCDD during organogenesis at doses of ~0.125 to 0.25 μg/kg produced hemorrhage of internal organs and subcutaneous edema (Sparschu et al. 1971a,b; Khera and Ruddick 1973). No effects were observed at 0.3 and 0.03 μg/kg, respectively, in NMRI mice and Sprague-Dawley rats. These teratogenic effects have been confirmed by a number of studies in which 2,3,7,8-TCDD was administered subcutaneously (studies summarized in EPA 1985b).

It has been demonstrated that both genetic susceptibility and concomitant exposure to other compounds affect the developmental toxicity of 2,3,7,8-TCDD. Poland and Glover (1980) and Dencker and Pratt (1981) demonstrated genetic differences in the susceptibility of mice; only responsive C57Bl/6J mice developed the characteristic cleft palate and hydronephrotic kidneys after treatment. This indicates that developmental toxicity, in addition to many other toxicological end points of 2,3,7,8-TCDD, segregates with the Ah locus. Additionally, it was demonstrated that simultaneous exposure to 2,3,7,8-TCDD and specific polychlorinated biphenyls (Birnbaum et al. 1985), or to the hormones hydrocortisone (Birnbaum et al. 1986) or thyroxine and triiodothyroxine (Lamb et al. 1986), increases the sensitivity of mice to the developmental effects of 2,3,7,8-TCDD. An additive effect was observed by Weber et al. (1985) for simultaneous exposure to 2,3,7,8-TCDD and 2,3,7,8-tetrachlorodibenzofurans (TCDF).

4.3.3.3 Dermal

Human. The EPA (1985a) evaluated several epidemiology studies of human populations exposed to herbicides contaminated with 2,3,7,8-TCDD.

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It is assumed that the exposure in these studies was predominantly dermal; however, some oral and inhalation exposure was also likely. The studies reviewed were predominantly geographic correlation studies that reported elevated incidence of birth defects, including cleft palate, cleft lip, neural-tube defects, heart abnormalities, hypospadias and epispadias, talipes, and cystic kidney disease, as well as increases in stillbirths (EPA 1979, Hanify et al. 1981, Field and Kerr 1979). Other similar studies reviewed failed to demonstrate a correlation between birth defects and possible exposure to 2,3,7,8-TCDD (Nelson et al. 1979, Thomas 1980, Dept. of Health New Zealand 1980, McQueen et al. 1977, Aldred 1978, Smith et al. 1982, Bonaccorsi et al. 1978, Reggiani 1980, Bisanti et al. 1980). In addition, a case control study of the offspring of Vietnam veterans potentially exposed to 2,3,7,8-TCDD in Agent Orange detected increases in birth defects that included spina bifida, cleft palate, and certain congenital tumors (Erickson et al. 1984). When all types of defects were combined, there was no elevated risk, and authors noted that the seemingly higher risk for individual birth defects may have resulted from chance or other unidentified risk factors. As a result of the inherent uncertainty in interpreting geographic correlation studies, the concomitant exposure to other potentially active compounds, and the review of similar studies that provided negative results, it was concluded that these investigations could neither prove nor disprove the hypothesis that 2,3,7,8-TCDD was a teratogen in humans.

Animal. No studies are available.

4.3.3.4 General discussion

The lack of a clearly defined exposed population, with adequate quantitative data on levels and duration of exposure, and the confounding presence of exposure to other chemicals that may in themselves be developmental toxicants, have made results of epidemiology data difficult to evaluate and inadequate for determining whether 2,3,7,8-TCDD induces developmental defects in humans. As with many developmental toxicants, the animal data indicated that the time of exposure was critical for the induction of effects, with treatment on days 8 or 11 producing maximal effects and treatment after 13 days being ineffective in mice (Neubert et al. 1973). Additional uncertainties regarding quantitative data for the effects of 2,3,7,8-TCDD on development result from the lack of experimental data for the guinea pig, which is known to be extremely sensitive to the acute toxic effects of 2,3,7,8-TCDD, and for the monkey in which 2,3,7,8-TCDD is a potent fetotoxic agent.

4.3.4 Reproductive Toxicity

4.3.4.1 Inhalation

Pertinent data regarding the reproductive toxicity of 2,3,7,8-TCDD in humans or animals following inhalation exposure were not found in the available literature.

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4.3.4.2 Oral

Human. No studies are available.

Animal. The fetotoxicity of 2,3,7,8-TCDD has been demonstrated following short-term exposure in utero (rats and mice) as well as in a multigeneration study (rats). Fetal death and vaginal bleeding have been observed in developmental toxicity studies [reviewed by EPA (1985a)] at doses of between 2 and 9 μg/kg/day. The three-generation study of Murray et al. (1979) reported that dietary administration of 2,3,7,8-TCDD at 0.01 and 0.1 μg/kg/day resulted in adverse effects on litter size, fetal survival, and neonatal survival in Sprague-Dawley rats. The 0.1-μg/kg/day dose also resulted in a significant decrease in fertility. Murray et al. (1979) considered the low dose of 0.001 μg/kg/day to be without substantial effects, since the only effect observed was an increase in dilated renal pelvises in the F1 generation. This effect was not observed at statistically significant levels in any other generation. The absence of effects at this lower dose level has been questioned by Nisbet and Paxton (1982), who reevaluated the data statistically and concluded that gestational index, decreased fetal weight, and the incidence of dilated renal pelvis were all increased at both the 0.01- and 0.001-μg/kg/day doses. The analysis by Nisbet and Paxton (1982) indicates that reproductive performance and the fetus are very sensitive to the toxic properties of 2,3,7,8-TCDD.

The monkey, however, appears more sensitive to the toxicity of 2,3,7,8-TCDD than either rats or mice. McNulty (1984, 1985) described the common occurrence of abortion in rhesus monkeys treated with a total of 1 μg/kg of 2,3,7,8-TCDD over days 20 to 40 of gestation; and the lowest total dose tested, 0.2 μg/kg, produced abortion in 1 of 4 test monkeys. In fetuses examined, there was either only minimal indication (palatal abnormalities) or no indication of developmental toxicity. In an earlier feeding study, groups of eight monkeys were maintained on diets containing 50 or 500 ppt (the total dose ingested was 1.8 and 11.7 μg, respectively) of 2,3,7,8-TCDD for 7 months prior to pregnancy and during pregnancy (Allen et al. 1979). In both groups, two-thirds of the pregnancies ended in abortion.

As reported in EPA (1985a), chronic exposure of rats to 2,3,7,8-TCDD in the diet results in a decrease in the weight of male reproductive organs. It was suggested by Moore et al. (1985) that this effect on the reproductive organs might account for the reduced male Wistar rat reproductive performance following 2,3,7,8-TCDD exposure, which had been earlier observed by Khera and Ruddick (1973). Levels of circulating androgens in 2,3,7,8-TCDD-treated male rats were studied by Moore et al. (1985) and shown to be depressed as a result of treatment. It was speculated that these depressed hormone levels may participate in the reproductive dysfunction in males. Circulating estradiol levels in pregnant females, however, appear not to be affected by 2,3,7,8-TCDD exposure, although 2,3,7,8-TCDD does effect some estrogen-metabolizing pathways when studied in vitro (Shiverick and Muther 1983).

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4.3.4.3 Dermal

Human. Epidemiology studies have been performed on groups of individuals exposed to herbicides or industrial chemicals contaminated with 2,3,7,8-TCDD. Although the dermal route is considered to be the predominant route of exposure in these studies, some exposure by other routes would also occur. These studies, which include those reviewed by EPA (1985a) and Friedman (1984) and that of Forsberg and Nordstrom (1985), were conducted in groups of male herbicide application workers, chemical plant employees, and soldiers exposed to Agent Orange in Vietnam, and to both male and females exposed through major industrial accidents or inappropriate disposal, which permitted the escape of large amounts of chemical from production plants. Studies of these groups have not clearly demonstrated that 2,3,7,8-TCDD produced any adverse effects on reproductive performance, although as a result of the limitations of the study, particularly with regard to the extent of exposure, it is not possible to interpret the results as indicative of the absence of reproductive toxicity for 2,3,7,8-TCDD in humans.

Animal. No studies are available.

4.3.4.4 General discussion

The evidence that 2,3,7,8-TCDD is a reproductive toxicant in humans is limited by the lack of exposure data and the concomitant exposure to other chemicals that may have been biologically active. The greatest exposure to 2,3,7,8-TCDD occurred in male herbicide sprayers, soldiers, and chemical plant workers, whereas the studies conducted have been concerned mostly with fetotoxicity and spontaneous abortion. Although the possibility of pre-mating exposure in males resulting in fetotoxicity and abortion is of concern, this end point is difficult to assess, particularly when the exposure is temporally removed from the time of conception. In addition, there is a lack of data on the functioning of male reproductive organs during the time of potential exposure to 2,3,7,8-TCDD. There are no equivalent female populations studied that have had extended periods of high exposure to chemicals contaminated with 2,3,7,8-TCDD, and only limited populations [females in the vicinity of Seveso (Bisanti et al. 1980, Bonaccorsi et al. 1978, Reggiani 1980) in the period immediately after the accident] from which to assess effects on reproduction in females exposed while pregnant.

Given the above limitations, the present epidemiology studies are only consistent with observations in animals that 2,3,7,8-TCDD elicits adverse effects on reproduction; they do not provide sufficient evidence to prove that this chemical is a reproductive toxicant. Animal studies indicate that 2,3,7,8-TCDD is a powerful reproductive toxicant; however, differences in species sensitivity have been observed. The available data indicate that the monkey may be the most sensitive species, but tests in this species have been limited to rhesus monkeys, with only a few animals studied at any dose level, which provides insufficient data to fully evaluate the range and extent of potential toxic effects in this species. In addition, the sensitivity of humans compared to monkeys, rats, or mice can only be speculated.

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4.3.5 Genotoxicity

4.3.5.1 Human

Although there have been no studies of human populations exposed to 2,3,7,8-TCDD alone, there are a number of studies on human populations exposed to chemicals contaminated with 2,3,7,8-TCDD, as reviewed in EPA (1985a). Czeizel and Kiraly (1976) reported that there was an increase in chromosomal aberrations of peripheral lymphocytes from workers in an East European 2,4,5-trichlorophenoxyethanol plant. Studies of soldiers (spouses and children of soldiers) exposed to Agent Orange (Mulcahy 1980) and of individuals involved in the Seveso accident (Reggiani 1980, Mottura et al. 1981, DiLernia et al. 1982, Tenchini et al. 1983, Kaye et al. 1985) have, however, failed to detect chromosomal aberrations. Some of the individuals in these studies had skin eruptions consistent with exposure to 2,3,7,8-TCDD. The only chromosomal effect in the latter studies was a decrease in satellite association (SA), which is evidence of functional ribosomal genes, in 2,3,7,8-TCDD-exposed subjects (DiLernia et al. 1982). The authors noted that similar decreases observed after x-irradiation exposure may possibly represent damage to the nucleolar organizing regions. All human data on chromosomal aberrations were confounded by mixed exposure to other potentially active compounds and the inability to determine quantitatively the extent of exposure to 2,3,7,8-TCDD.

4.3.5.2 Nonhuman

A recent review by Giri (1986) of the mutagenic and genotoxic effect of 2,3,7,8-TCDD concluded that there is evidence for the genotoxic activity of 2,3,7,8-TCDD, but additional testing would be required to demonstrate this activity with certainty. The results of nonhuman genotoxicity studies are summarized in Tables 4.2 and 4.3. The early positive results of Hussain et al. (1972) and Seiler (1973) are likely artifacts resulting from extensive cell death and possibly from impurities in the test material. In vitro cytogenic tests in yeast in both the standard plate test and the intrasanguineous host-mediated assay (using CD-1 mice) have produced positive results (Bronzetti et al. 1983). In in vivo cytogenetic assays, Loprieno et al. (1982) observed that the positive responses depended on sampling time, with negative results obtained 24 h posttreatment and positive results 96 h posttreatment. Although the toxic properties of 2,3,7,8-TCDD usually segregate with the Ah locus, Meyne et al. (1985) observed negative responses in vivo after administration of 2,3,7,8-TCDD at hepatotoxic levels to both responsive, C57Bl/6J, and nonresponsive, DBA/2J, mice when sampled at 24 h. There are also limited supportive data for the mutagenicity of 2,3,7,8-TCDD from the observation in vivo of the low-level binding of 2,3,7,8-TCDD to liver macromolecules (Poland and Glover 1979).

4.3.5.3 General discussion

The nonhuman genotoxicity data on 2,3,7,8-TCDD are conflicting; negative results were reported in many of the assay systems, and when positive results were observed, the response was generally weak. These

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Table 4.2. Genotoxicity of 2,3,7,8-TCDD in vitro

End point	Species (test system)	Results with activation/without activation	References
Gene mutation	*Salmonella typhimurium* (reverse mutation)	−/−	McCann 1978, Gilbert et al. 1980, Geiger and Neal 1981, Mortelmans et al. 1984
	S. typhimurium (reverse mutation)	Not tested/+	Hussain et al. 1972, Seiler 1973
	Escherichia coli (reverse mutation)	Not tested/+	Hussain et al. 1972
	Saccharomyces cerevisiae (reversion)	+/−	Bronzetti et al. 1983
	L5178Y mouse lymphoma cells (forward mutation)	Not tested/+, and not tested/−	Rogers et al. 1982
Cytogenetic	*S. cerevisiae* (gene conversion)	+/−	Bronzetti et al. 1983
	S. cerevisiae (host mediated)	+/NA[a]	Bronzetti et al. 1983
	Chinese hamster cells (sister chromatid exchange)	Not tested/−	Toth et al. 1984
Cell transformation	Baby hamster kidney cells - BHK	Not tested/+	Hay 1982
	C3H/10 T1/2 cells	Not tested/−	Abernathy et al. 1985

[a]Not available.

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Table 4.3. Genotoxicity of 2,3,7,8-TCDD in vivo

End point	Species (test system)	Results	References
Gene mutation	*Drosophila* (sex-linked recessive lethal)	−	Zimmering et al. 1985
Cytogenetic	*Drosophila* (sister chromatid exchange)	−	Zeiger 1983
	Drosophila (structural aberration)	−	Zeiger 1983
	Rat (sister chromatid exchange)	−	Lundgren et al. 1986
	Rats - marrow cells (structural aberration)	−	Green and Moreland 1975
	Rats - marrow cells (structural aberration)	+	Green et al. 1977
	Mouse - marrow cells (structural aberration)	+	Loprieno et al. 1982
	Mouse - marrow cells (sister chromatid exchange)	−	Meyne et al. 1985
	Mouse - marrow cells (structural aberration)	−	Meyne et al. 1985
	Mouse - marrow cells (micronucleus)	−	Meyne et al. 1985

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conflicting data may result from technical difficulties in testing 2,3,7,8-TCDD rather than from a lack of biological activity. Testing difficulties arise from the extreme insolubility of this compound and the high toxicity observed in some test systems, which would be anticipated to result in a very narrow window for effective genotoxic doses. As a result of the largely negative data from nonhuman genotoxicity assays, some investigators, as discussed by Paustenbach et al. (1986), have concluded that 2,3,7,8-TCDD is not a genotoxic agent. Sufficient data are not available to precisely define the toxicological mechanism of 2,3,7,8-TCDD and resolve these differences of opinion.

Human studies are primarily limited by the lack of data on the extent of exposure. The only indication of exposure in these studies was the development of gross skin lesions, which does not provide a good estimate of either the extent of exposure or the duration. In addition, the time after exposure that the cells were examined may not have been optimal for observing cytogenetic effects. Because of the limitations of the studies in humans, these data cannot be used to demonstrate that 2,3,7,8-TCDD does not pose a genotoxic hazard.

4.3.6 Carcinogenicity

4.3.6.1 Inhalation

Pertinent data regarding the carcinogenicity of 2,3,7,8-TCDD following inhalation exposure of humans or animals were not found in the available literature.

4.3.6.2 Oral, human.

No studies are available.

Animal. A number of bioassays have been conducted, and all have demonstrated that 2,3,7,8-TCDD is carcinogenic in animals via the oral route. The NTP (1982a) and Kociba et al. (1978a,b) studies are key bioassays of 2,3,7,8-TCDD. Although both studies used sufficient numbers of animals and exposure durations, the study by Kociba et al. (1978a,b) is relevant to human exposure scenarios because the compound was administered daily in the diet rather than biweekly by gavage as in the NTP (1982a) study. Furthermore, the study by Kociba et al. (1978a,b) has proven to be the most sensitive indicator of the carcinogenic potency of 2,3,7,8-TCDD. The details of the Kociba et al. (1978a,b) study are summarized in Table 4.4. Other oral studies which support the conclusion that 2,3,7,8-TCDD is an animal carcinogen are presented in Table 4.5.

4.3.6.3 Dermal

Human. Although there are no known cohorts that have been exposed solely to 2,3,7,8-TCDD, a number of cohorts that have been exposed to herbicides or industrial chemicals contaminated with 2,3,7,8-TCDD have been studied. The predominant exposure route for these cohorts was probably dermal, although some inhalation and oral exposure was also likely. In addition, 2,3,7,8-TCDD was only a minor contaminant of these compounds, and any effects observed may have been either caused or potentiated by the contaminated compound itself.

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Table 4.4 Summary of the oral carcinogenicity bioassay of Kociba et al. (1978a,b)

Animal	Sex	Dose tested (μg/kg/day)	Tumor type	Incidence
Sprague-Dawley rats	M	Control	Squamous cell carcinoma of the tongue, adenoma of the adrenal cortex, and squamous cell carcinoma of the hard palate	0/85
		0.001	Squamous cell carcinoma of the tongue	1/50
		0.01	Squamous cell carcinoma of the tongue	1/50
			Adenoma of the adrenal cortex	2/50
		0.1	Squamous cell carcinoma of the tongue	3/50
			Adenoma of the adrenal cortex	5/50
			Squamous cell carcinoma of the hard palate	4/50
	F	Control	Hepatocellular carcinoma	1/86
		0.001	Hepatocellular carcinoma	0/50
		0.01	Hepatocellular carcinoma	2/50
			Squamous cell carcinoma of the hard palate	1/50
		0.1	Hepatocellular carcinoma	11/49
			Squamous cell carcinoma of the hard palate	4/49
			Squamous cell carcinoma of the lung	7/49

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Table 4.5. Other oral studies supporting the conclusion that 2,3,7,8-TCDD is an animal carcinogen

Method of exposure	Animal	Sex/ number	Doses tested	Tumor type	References
Diet	Sprague-Dawley rats	M/10	0.01, 0.005, 0.05, 0.5, 1.0, or 5 ppb	Increase in total tumor incidence	Van Miller et al. 1977a,b
Gavage	Osborne-Mendel rats	M/50	0.01, 0.05, or 0.5 μg/kg/week	Follicular-cell adenomas and carcinomas of the thyroid	NTP 1982a
	Osborne-Mendel rats	F/50	0.01, 0.05, or 0.5 μg/kg/week	Neoplastic nodules and hepatocellular carcinomas of the liver	NTP 1982a
	B6C3F1 mice	M/50	0.01, 0.05, or 0.5 μg/kg/week	Hepatocellular carcinomas	NTP 1982a
	B6C3F1 mice	F/50	0.01, 0.05, or 0.5 μg/kg/week	Hepatocellular carcinoma and follicular-cell adenomas of the thyroid	NTP 1982a
	Swiss mice	M/44	0.007, 0.7, or 7.0 μg/kg/week	Hepatomas and hepatocellular carcinomas	Toth et al. 1979

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EPA (1985a) reviewed several epidemiology studies of humans exposed to herbicides contaminated with 2,3,7,8-TCDD. The studies by Axelson et al. (1980) and Thiess and Frentzel-Beyme (1977) found an association between exposure and stomach cancer, and a series of other studies (Hardell and Sandstrom 1979; Eriksson et al. 1979, 1981; Hardell et al. 1980, 1981; Lynge 1985; Palovi et al. 1986) reported an association between exposure and soft-tissue sarcomas (of various sites) and lymphomas. Other studies, however, reviewed by EPA (1985a) and Hiremath et al. (1986), failed to detect an association between herbicide exposure and the induction of cancer (Ott et al. 1980; Zack and Suskind 1980; Cook et al. 1980, 1985; Cook 1981; Pazderova-Vejlupkova et al. 1981; Johnson et al. 1981; Riihimaki et al. 1982; Smith et al. 1983; Wolfe et al. 1984; Smith and Pearce 1985). Fingerhut et al. (1984), Cook et al. (1986), and Ott et al. (1987) also did not detect an association between herbicide exposure and cancer. The extent of exposure to 2,3,7,8-TCDD in some of the epidemiology studies is questionable, and precise quantification of exposure is not available for all of the studies.

Animal. There is only limited evidence that 2,3,7,8-TCDD produces tumors in laboratory animals following dermal exposure. NTP (1982b) administered 2,3,7,8-TCDD alone to Swiss mice. Female mice, but not male mice, had an increased incidence of fibrosarcomas in the integumentary system.

As reviewed by EPA (1985a), there is conflicting evidence that 2,3,7,8-TCDD acts as a tumor promotor when applied to the skin. Berry et al. (1978) failed to detect any tumor-promoting activity by 2,3,7,8-TCDD in CD-1 mice skin initiated with dimethylbenzanthracene (DMBA), and Slaga and Nesnow (1985) reported that unpublished data indicated that 2,3,7,8-TCDD either had no promoting activity or very weak promoting activity in Sencar mice skin. NTP (1982b) also examined the ability of 2,3,7,8-TCDD to act as a tumor promoter in Swiss Webster mice, and animals treated with 2,3,7,8-TCDD alone had similar tumor incidences to animals treated first with DMBA followed by treatment with 2,3,7,8-TCDD. NTP (1982b) concluded, however, that "in the DMBA-TCDD experiment, failure to have included groups skin painted with only DMBA precludes interpretation of these results." Poland et al. (1982), however, not only demonstrated promotion, but also that genetic differences in CD-1 mice affect the ability of 2,3,7,8-TCDD to act as a promotor. In HRS/J mice homozygous for the hairless trait, promotion with 2,3,7,8-TCDD after initiation with DMBA produced as many skin tumors (both incidence and multiplicity) as promotion with the known promotor TPA; whereas in mice heterozygous for the hairless trait (and wild type), skin tumors could only be promoted by TPA but not 2,3,7,8-TCDD. Even in the homozygous mice, 2,3,7,8-TCDD did not produce the commonly observed hyperplasia associated with promotors such as TPA, suggesting that 2,3,7,8-TCDD has a different mechanism of action. Other studies indicated that pretreatment with 2,3,7,8-TCDD could block the subsequent DNA binding of known carcinogens and also prevent tumor initiation (Berry et al. 1979, Cohen et al. 1979). Although not a dermal study, Pitot et al. (1980) also demonstrated promotion by administering a single intragastric dose of the hepatocarcinogen diethylnitrosamine (DEN) followed by repeated subcutaneous injections of 2,3,7,8-TCDD. No

hepatic tumors were observed in animals given DEN or 2,3,7,8-TCDD alone, but the combined treatment resulted in the development of hepatocellular carcinomas.

4.3.6.4 General discussion

The evidence from human epidemiology studies that 2,3,7,8-TCDD is carcinogenic is difficult to assess because (1) exposure to 2,3,7,8-TCDD is poorly documented and (2) exposure occurred to other potentially active materials. The strongest evidence is from the induction of soft-tissue sarcomas at various sites, and it has been questioned whether combining tumor data from various sites is appropriate. With regard to the observed increase in stomach cancer, the two groups of workers studied were relatively small, and similar increases in stomach tumors have not been reported in other more extensive studies.

The animal data, however, clearly indicate that 2,3,7,8-TCDD is carcinogenic, although there is some disagreement in the scientific and international regulatory community as to whether 2,3,7,8-TCDD acts as a complete carcinogen or as a carcinogen promotor (Shu et al. 1987). The rationale for describing 2,3,7,8-TCDD as a promotor is based on the poor response of 2,3,7,8-TCDD in many short-term mutagenicity assays, the lack of strong evidence for binding to DNA, and positive results in in vivo promotion assays using the classic skin painting technique (as described above) and the study of Pitot et al. (1980) using the two-stage model in rat liver. The classic feeding study bioassays, however, would support the view that 2,3,7,8-TCDD is a complete carcinogen.

4.4 INTERACTIONS WITH OTHER CHEMICALS

There are few data on the interactions of 2,3,7,8-TCDD with other chemicals. As discussed in EPA (1985a), 2,3,7,8-TCDD is a strong inducer of microsomal enzymes; hence, prior exposure to 2,3,7,8-TCDD will alter the rate of metabolism and toxicity of many compounds that are either detoxified or activated by this enzyme system. The observed inhibition by 2,3,7,8-TCDD of the tumorigenic response of known tumorigens in the mouse skin bioassay (Berry et al. 1979, Cohen et al. 1979) may be an example of 2,3,7,8-TCDD-induced enzyme changes altering the metabolic fate and toxicity of another compound.

The only other interactions that have been observed are the additive effect of 2,3,7,8-TCDD and similar polychlorinated dibenzofurans and polychlorinated biphenyls on the induction of cleft palate in mice (Weber et al. 1985, Birnbaum et al. 1985), and the increased sensitivity of mice to 2,3,7,8-TCDD-induced cleft palate with the co-administration of the hormones thyroxine and triiodothyroxine (Lamb et al. 1986). With regard to environmental exposure, the relevance of interactions of 2,3,7,8-TCDD and the high levels of hormones used in the latter study is unclear.

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5. MANUFACTURE, IMPORT, USE, AND DISPOSAL

5.1 OVERVIEW

2,3,7,8-TCDD is neither commercially manufactured nor imported into the United States. It is produced inadvertently in small amounts as an impurity during the manufacture of compounds for which 2,4,5-trichlorophenol is a synthetic intermediate. At the present time, its only use is in chemical research. Several field-tested and untested methods are available for the disposal of 2,3,7,8-TCDD-containing wastes. Some of the promising methods are incineration at high temperature, oxidative destruction with the aid of a catalyst, biodegradation by a fungus, photochemical destruction in the presence of a hydrogen-donating substrate, and stabilization (in case of soils) through the in situ addition of cementitious and asphaltic materials.

5.2 PRODUCTION

2,3,7,8-TCDD is synthesized on a laboratory scale primarily by two processes: (1) condensation of dichlorocatechol with substituted dichlorobenzenes and (2) halogenation of dibenzo-p-dioxin or its dichloro-substituted derivative (EPA 1985b). 2,3,7,8-TCDD is not commercially manufactured in the United States but is produced as an undesirable product during the manufacture of compounds for which 2,4,5-trichlorophenol is a synthetic intermediate (see Sect. 6.2 on Releases to the Environment).

5.3 IMPORT

2,3,7,8-TCDD is not imported into the United States (EPA 1984b).

5.4 USE

2,3,7,8-TCDD has been tested for flame-proofing polyesters and as a control against insects and wood-destroying fungi in Germany; however, it has probably never been commercially produced or used for these purposes. At present it is only used as a research chemical (HSDB 1987).

5.5 DISPOSAL

For the disposal of wastes and residues containing 2,3,7,8-TCDD, incineration at a minimum temperature of 800 to 1200°C and a contact time of >30 s was found to be satisfactory. Polychlorophenols containing 2,3,7,8-TCDD as an impurity have been disposed of by dispersing or dissolving the material in water, or a nonnucleophilic organic solvent, and oxidizing with ruthenium tetraoxide catalyst at ~70°C (HSDB 1987). Satisfactory field-tested methods for the disposal of 2,3,7,8-TCDD-containing soils were not available until recently. The following methods have either shown promise or are presently used for the disposal

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of 2,3,7,8-TCDD: (1) stabilization through the in situ addition of cementitious and asphaltic materials; (2) degradation with *Phanerochaete chrysosporium*, a white rot fungus; (3) a mobile incineration system for the thermal destruction of 2,3,7,8-TCDD; and (4) ultraviolet photolysis in the presence of alkali polyethylene glycolate reagents (des Rosiers 1986). A method (similar to method 4) for photochemical destruction of 2,3,7,8-TCDD in the presence of olive oil (a hydrogen donor) was used with soil from the area of the Seveso accident. A recent report discusses the disposal of 2,3,7,8-TCDD by a mobile poly(ethylene)glycol-potassium hydroxide destruction unit (Rogers et al. 1987).

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6. ENVIRONMENTAL FATE

6.1 OVERVIEW

The important sources of 2,3,7,8-TCDD in the environment are production and use of certain herbicides and chlorophenols, incineration of municipal and industrial wastes, and improper disposal of chemical wastes produced during the manufacture of 2,4,5-trichlorophenol, 2,4,5-T, and related herbicides, hexachlorophene, and chlorinated benzenes. The fate of 2,3,7,8-TCDD in the environment is not clearly understood. It appears that particulate-bound 2,3,7,8-TCDD in the air may undergo photolysis and may be removed by wet and dry deposition. The half-life of atmospheric 2,3,7,8-TCDD is such that 2,3,7,8-TCDD can be transported long distances in the air. The ultimate sink of airborne 2,3,7,8-TCDD is sediments of surface waters. The two processes that are likely to remove 2,3,7,8-TCDD from water and soils are vaporization and photolysis. The estimated half-life of 2,3,7,8-TCDD in surface water is >1 year, and the ultimate sink of aquatic 2,3,7,8-TCDD is sediments. The bioconcentration factor of 2,3,7,8-TCDD in the fathead minnow (*Pimephales promelas*) is 7900 to 9300. 2,3,7,8-TCDD is immobile in most soils, but horizontal movement of soil-bound 2,3,7,8-TCDD may occur in runoff water during flooding. As observed in Seveso, Italy, minimal vertical movement may occur in soils containing low organic matter. The estimated half-life of 2,3,7,8-TCDD is 1 to 3 years on soil surfaces and 10 to 12 years in the interior of soils. Although not accumulated, the level of 2,3,7,8-TCDD absorbed in parts of plants underground is of the same order of magnitude as in soil, but the aerial parts of plants contain 50% lower concentrations.

6.2 RELEASES TO THE ENVIRONMENT

Although the following paragraphs discuss the sources of 2,3,7,8-TCDD in the environment, the sources responsible for its background levels are not clear.

6.2.1 Production and Use of Certain Herbicides and Chlorophenols

The phenoxy herbicide 2,4,5-T produced prior to 1960 contained up to 100 μg/g 2,3,7,8-TCDD. The level of 2,3,7,8-TCDD in commercial 2,4,5-T has been reduced in recent years to <0.1 μg/g, and most commercial 2,4,5-T available today may contain <0.02 μg/g 2,3,7,8-TCDD. Agent Orange, a 1:1 mixture of butyl esters of 2,4,5-T and 2,4-D produced before 1970, contained 0.02 to 54 μg/g 2,3,7,8-TCDD. Hexachlorophene, a germicide manufactured from trichlorophenol, contains 0.2 to 0.5 ng/g 2,3,7,8-TCDD. 2,4,6-Trichloro-, 2,3,4,6-tetrachloro-, and pentachlorophenol were found to contain <0.1 μg/g other tetra isomers but no 2,3,7,8-TCDD. 2,3,7,8-TCDD was detected at a concentration <1 ng/g (2,3,7,8-TCDD detection limit of 0.03 ng/g) in all samples of sodium pentachlorophenate, 2,3,4,5-tetrachlorophenol, and

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hexachlorophene. 2,4,5-Trichlorophenol, on the other hand, contained up to 6.2 μg/g 2,3,7,8-TCDD. Similarly, diphenyl ether herbicides were found to contain other tetrachloro isomers but no 2,3,7,8-TCDD (EPA 1985b, HSDB 1987, Rappe 1984, Hagenmaier 1986, Weeren and Asshauer 1985). From the analysis of sediments of a western Lake Ontario site, Czuczwa and Hites (1986) concluded that the likely source of tetrachlorodibenzo-p-dioxins was a pentachlorophenol production facility. The analytical method used, however, could not distinguish 2,3,7,8-TCDD from other tetra isomers.

6.2.2 Photochemical Reactions

The photochemical reaction of phenoxy herbicides has been found to produce polychlorinated dibenzo-p-dioxins through photodechlorination and subsequent condensation reactions; however, this process does not produce 2,3,7,8-TCDD (Rappe 1984). Lower substituted dibenzo-p-dioxins are also formed during photodechlorination of higher chlorine-substituted dibenzo-p-dioxins. Trace amounts of 2,3,7,8-TCDD were observed from the photodechlorination of both 1,2,3,6,7,8-hexa- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (Buser 1979).

6.2.3 Thermal Reactions

Small amounts of 2,3,7,8-TCDD have been detected in the flue gases from municipal incinerators. From the experimentally determined concentrations in flue gases of five municipal incinerators, the maximum average concentration of 2,3,7,8-TCDD in ambient air at ground level was estimated as 38 fg/g. Incineration of industrial wastes containing 2,4,5-T salts and esters, polychlorinated benzenes, and chlorophenoxy ethers also produced 2,3,7,8-TCDD (Rappe 1984, Barnes 1983). Upon analysis of sediments from Saginaw Bay, Saginaw River, and the Great Lakes, Czuczwa and Hites (1984, 1986) concluded that the source of tetrachlorodibenzo-p-dioxins was incineration, although the analytical method used was unable to separate 2,3,7,8-TCDD from other tetra isomers. Combustion of coal did not produce 2,3,7,8-TCDD at a detection limit of 1.2 ng/kg (HSDB 1987), but burning of woods did produce 0.65 μg/kg 2,3,7,8-TCDD (EPA 1985b). Exhausts from automobiles powered with leaded gasoline were reported to contain <0.05 to 0.3 ng 2,3,7,8-TCDD/24.8 km, but no 2,3,7,8-TCDD was detected in exhausts of automobiles powered with unleaded gasoline (Marklund et al. 1987). Accidental fires involving capacitors or transformers containing chlorobenzene will also release 2,3,7,8-TCDD to the environment. An example of such a contamination is the State Office Building in Binghamton, New York.

6.2.4 Improper Disposal of Chlorinated Chemical Wastes

Improper disposal of certain chemical wastes produced during the manufacture of 2,4,5-trichlorophenol, 2,4,5-T, and related herbicides, hexachlorophene, chlorinated benzenes, etc., may be a source of 2,3,7,8-TCDD in the environment. Examples of such improper disposal leading to the contamination of the environment are the Love Canal, Niagara Falls, New York, sites where 2,3,7,8-TCDD up to a level of 672 μg/kg was detected. Similarly, several sites in the state of Missouri were contaminated with up to 1750 μg/kg 2,3,7,8-TCDD (Tiernan et al.

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1985).

6.3 ENVIRONMENTAL FATE

The fate of 2,3,7,8-TCDD in air, water, and soil is not understood with certainty. Although some experimental efforts have been directed in recent years to elucidate its fate in different media, a substantial data gap exists in this area. In air, 2,3,7,8-TCDD is likely to be present predominantly in the gas phase. The two important processes that may remove 2,3,7,8-TCDD from the atmosphere are photochemical degradation and wet deposition. Even an estimate of the atmospheric half-life of 2,3,7,8-TCDD is not available. On the basis of photochemical experiments with 2,3,7,8-TCDD coated on silica gel, the half-life of atmospheric particulate 2,3,7,8-TCDD may be a few days. The half-life of atmospheric gas-phase 2,3,7,8-TCDD may be higher than particulate 2,3,7,8-TCDD. The lifetime of atmospheric 2,3,7,8-TCDD is such that it can be transported long distances in the air. The ultimate environmental sink of airborne particulate 2,3,7,8-TCDD is likely to be sediments of surface waters (Eitzer and Hites 1986, Czuczwa and Hites 1986, Choudhry and Hutzinger 1982).

The biodegradation of 2,3,7,8-TCDD in water is probably slow. The two processes that may be important for the removal of 2,3,7,8-TCDD are volatility and photodegradation. Although the photolysis of 2,3,7,8-TCDD in hydrogen-donating solvents is a fast process, a suspension of 2,3,7,8-TCDD in distilled water showed no appreciable photodegradation. In natural waters, the presence of small amounts of hydrogen-donating substrate or the presence of photosensitizers may account for its observed photodegradation; however, the photochemical degradability of 2,3,7,8-TCDD in water, as provided by model ecosystem studies (Tsushimoto et al. 1982, Matsumura et al. 1983), has not provided definite evidence through mass balance that the observed loss of 2,3,7,8-TCDD attributed to photolysis was not due to its sorption on sediment and biota. The photodegradation is usually a dechlorination process leading to the formation of tri- and dichlorinated dibenzo-p-dioxins. In sediment-containing lake water, the estimated half-life of 2,3,7,8-TCDD is >1.5 years. In lake water alone, the estimated half-life is >1 year. The ultimate sink of aquatic 2,3,7,8-TCDD is the sediment. Recent flow-through experiments with fathead minnows (*Pimephales promelas*) have shown that the bioconcentration factor for 2,3,7,8-TCDD in this species is 7900 to 9300 on a wet weight basis (EPA 1985b, Adams et al. 1986).

2,3,7,8-TCDD is expected to be immobile in most soils by irrigation and rainfalls. A downward movement of 10 cm in 12 years was observed with soil from Eglin Air Force Base. Although 2,3,7,8-TCDD usually does not leach through soil, leaching is possible in rare instances from soils of very low organic carbon content as a result of 2,3,7,8-TCDD solvation with organic solvent or biotic mixing by earthworms or other soil invertebrates. A white rot fungus (*Phanerochaete chrysosporium*) has been shown to degrade 2,3,7,8-TCDD. This biodegradation does not occur significantly in natural soils, probably because of the lack of this or other degrading microorganisms. Both volatilization and photoreaction may remove some 2,3,7,8-TCDD from soil surfaces. The photoreaction on soil surfaces can be greatly enhanced by the presence of hydrogen-

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donating substrates (e.g., olive oil or arachis oil) in soil. The photoreaction will be insignificant beyond the surface soil layers. The estimated half-life of 2,3,7,8-TCDD on soil surfaces is 1 to 3 years, but the half-life in the interior of soil may be 10 to 12 years (EPA 1985b, Freeman and Schroy 1986, Bumpus et al. 1985, HSDB 1987).

2,3,7,8-TCDD present on leaves of plants as a result of spraying herbicides will photolyze with a half-life of a few hours. The chemical is absorbed by higher plants and is probably translocated, but it is not accumulated. The absorption by underground parts may be at the same level as soil, but the aerial part contains ~50% lower concentrations (Choudhry and Hutzinger 1982, Sacchi et al. 1986).

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7. POTENTIAL FOR HUMAN EXPOSURE

7.1 OVERVIEW

The concentration of total tetrachlorodibenzo-p-dioxin (not 2,3,7,8-TCDD alone) in ambient air in Bloomington, Indiana, was 18 to 92 fg/m^3 (femtograms per cubic meter). Assuming that the 2,3,7,8-TCDD isomer constitutes 5% of the total tetra isomers, the concentration of 2,3,7,8-TCDD in Bloomington air would be 0.9 to 4.6 fg/m^3. The concentration of 2,3,7,8-TCDD in the air around the stack of a municipal incinerator has been estimated as 38 fg/m^3. The accidental transformer fire in Binghamton, New York, produced a much higher level of 2,3,7,8-TCDD--at 0.23 to 0.47 pg/m^3. The concentration of 2,3,7,8-TCDD in the air surrounding a field after the application of Silvex containing 15 ppm 2,3,7,8-TCDD was 0.62 pg/m^3. Other than certain industrial effluents and leachates from chemical dump sites, no 2,3,7,8-TCDD has ever been reported in drinking water. The concentrations of 2,3,7,8-TCDD in most uncontaminated soils are below the detection limits of analytical methods. In urban soils, the level of 2,3,7,8-TCDD is in the range <0.0002 to 0.009 ng/g. Much higher levels have been detected in soils contaminated by certain hazardous wastes, waste oils, and spillage of 2,4,5-trichlorophenol. A soil from Shenandoah Stables in Missouri contaminated by waste oil containing 2,3,7,8-TCDD had up to 1750 ng/g of 2,3,7,8-TCDD. Fish samples collected from lakes and a selected Michigan river contained undetectable levels to 67 pg/g 2,3,7,8-TCDD. No 2,3,7,8-TCDD was detected in rice, soybeans, and crawfish in the United States, or in Canadian chicken and pork samples. Since fat is the chief contributor to the body burden of 2,3,7,8-TCDD in humans, it has been analyzed by many investigators. Levels of 2,3,7,8-TCDD in adipose tissue of exposed and control persons in Missouri have been determined. The level of 2,3,7,8-TCDD in adipose tissue in the general population in the United States and Canada ranges from undetectable to 20 pg/g, with a mean value of 5 to 7 pg/g. Values as high as 99 pg/g were detected in an individual heavily exposed during the spraying operation in South Vietnam. 2,3,7,8-TCDD has not been detected in human milk from exposed and control areas in the United States; however, using more sensitive methods of measurement, 2,3,7,8-TCDD has been reported in human milk of the general population in Sweden at trace to 2.3 pg/g, with a mean value of 0.6 pg/g, and in Germany at 1.3 to 3.3 pg/g, with a mean value of 1.9 pg/g. 2,3,7,8-TCDD was also found in human milk in other European countries. Although there are no data regarding exposure levels, workers at sites of improper chemical waste disposal and the general population residing near these sites may be susceptible to higher exposure to 2,3,7,8-TCDD. Breast-fed babies nursed by mothers residing near improperly controlled municipal incinerators may also be at higher risk. The estimated daily human exposure to 2,3,7,8-TCDD, expressed as picograms per kilogram of body weight (pg/kg), is 0.02 through inhalation, 0.5 to

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5 through the consumption of milk, and 20 through the consumption of fish. The daily exposure of a 5-kg baby resulting from the consumption of 850 mL of breast milk is 20 to 200 pg/kg. From the estimated body burden and the half-life of 2,3,7,8-TCDD in the human body, the daily human intake of 2,3,7,8-TCDD has been estimated as 0.05 ng.

7.2 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

7.2.1 Air

The level of 2,3,7,8-TCDD in ambient air is so low that its detection is beyond the capabilities of analytical methods. The concentration of total tetrachlorodibenzo-p-dioxin isomers in both vapor and particulate phase in the ambient air in Bloomington, Indiana, was approximately 18 to 92 fg/m^3. The analytical method used could not unequivocally identify 2,3,7,8-TCDD from other tetra isomers. Using the measured concentration in flue gases from five municipal incinerators and an air dispersion model, the maximum ambient concentration of 2,3,7,8-TCDD in the area around the stacks was estimated as 38 fg/m^3. Since municipal incinerators are one of the prime sources of atmospheric 2,3,7,8-TCDD, there are a vast number of publications concerning the level of these compounds in the fly ashes of the incinerators; however, most of the publications failed to distinguish 2,3,7,8-TCDD from its isomers. The levels of 2,3,7,8-TCDD in the flue gas of European incinerators are 0.05 to 1.3 ng/m^3; in U.S. incinerators, the levels are a maximum of 3.5 ng/m^3 (under normalized conditions of the emitted gases) (EPA 1985b, Barnes 1983, Nottrodt and Ballschmiter 1986, Marklund et al. 1986, Eitzer and Hites 1986).

Accidents involving certain transformer/capacitor fires can release larger amounts of 2,3,7,8-TCDD in the air. The concentration of 2,3,7,8-TCDD in the air of the State Office Building in Binghamton, New York, following an accidental fire, was 0.23 to 0.47 pg/m^3. Following an accidental locomotive fire in Sweden, the concentration of 2,3,7,8-TCDD was 50 pg/m^3. The concentration of 2,3,7,8-TCDD in the air surrounding a field after the application of Silvex containing 15 ppm 2,3,7,8-TCDD was 0.62 pg/m^3, but the concentration dropped to a level of 10 fg/m^3 in 200 days (EPA 1985b, Smith et al. 1986, Rappe et al. 1985).

7.2.2 Water

No report is available on the detection of 2,3,7,8-TCDD in drinking water, using methods with detection limits in the nanogram-per-liter range; however, 2,3,7,8-TCDD has been detected in aqueous industrial effluents, sediments, and leachates from hazardous waste sites. The concentrations of tetrachlorinated dibenzo-p-dioxins, including 2,3,7,8-TCDD, in effluents from a trichlorophenol manufacturing facility ranged from none detected (detection limit, 10 to 30 pg/g) to 100 pg/g. The discharged wastewater effluent from Dow into the Tittabawassee River, in Michigan, has been reported to be approximately 15 pg/L. The leachate samples from a waste disposal site in Jacksonville, Arkansas, had a mean 2,3,7,8-TCDD level of 14 ng/L. The sump pump water from residences and leachates from the Love Canal area in New York contained 2,3,7,8-TCDD ranging from none detected to 1560 ng/L. The concentrations of 2,3,7,8-TCDD in sediments from storm sewers, residential sump water,

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and surface water around the same site were none detected (detection limit, 10 to 100 pg/g) to 9570 ng/g (EPA 1985b, Tiernan et al. 1985, Lamparski et al. 1986).

7.2.3 Soil

Concentrations of 2,3,7,8-TCDD in most uncontaminated soils are below the detection limits of current analytical methods. In urban soils, the level of 2,3,7,8-TCDD is in the range of <0.0002 to 0.009 ng/g. 2,3,7,8-TCDD has been detected in samples that originated from certain industrial sites, waste disposal sites, and sites involved in accidental spillage of chemicals containing 2,3,7,8-TCDD. The levels of 2,3,7,8-TCDD in soils from different locations are given in Table 7.1. It is apparent from Table 7.1 that the accidental or improper disposal of still-bottom residue from the manufacture of 2,4,5-trichlorophenol (2,4,5-TCP) may be the largest source of 2,3,7,8-TCDD in soils.

7.2.4 Other

There are limited data that 2,3,7,8-TCDD does not bioaccumulate in crop plants (Anonymous 1985). Crops grown in soil contaminated with 2,3,7,8-TCDD (up to 752 ppt) after the Seveso accident contained only a few parts per trillion of 2,3,7,8-TCDD in the aboveground portions. The roots of these plants, however, contained higher levels of 2,3,7,8-TCDD than the surrounding soil, suggesting that a similar study using root crops would demonstrate the contamination of the edible portion of the plant.

Since aquatic organisms bioconcentrate 2,3,7,8-TCDD, a few investigators analyzed fish-eating birds as an indicator of possible pollution in the suspected water bodies. A herring gull sample from Lake Huron contained 75 pg/g 2,3,7,8-TCDD. Similarly, herring gull eggs collected from the Great Lakes contained 12 to 101 pg/g 2,3,7,8-TCDD. Samples from Lake Ontario and Saginaw Bay had the maximum levels of 2,3,7,8-TCDD; those from Lake Michigan and Lake Superior had the minimum levels of contamination (Stalling et al. 1986, Buser and Rappe 1984). Fish samples from the Great Lakes and selected Michigan rivers were shown to contain between undetectable amounts (detection limit, 2 pg/g) and 67 pg/g 2,3,7,8-TCDD (Fehringer et al. 1985, Niemann 1986, Ryan et al. 1984). Yellow perch samples from Woods Pond, Massachusetts, were found to contain 26 pg/g 2,3,7,8-TCDD (Buser and Rappe 1984). No 2,3,7,8-TCDD was found in rice, soybean, and crawfish samples from Arkansas and Louisiana at a detection limit of 10 pg/g, or in Canadian chicken and pork samples at a detection limit of 2 to 4 pg/g (Ryan et al. 1985a, Firestone et al. 1985).

Since adipose tissue is the chief contributor to the body burden of 2,3,7,8-TCDD, many investigators analyzed fat tissue from both exposed and control populations. That fat has the highest burden is confirmed by the tissue analysis of a woman who died 7 months after the accident in Seveso, Italy. The following levels of 2,3,7,8-TCDD (pg/g) were found in different organs: fat, 1840; pancreas, 1040; liver, 150; thyroid, 85; brain, 60; lung, 60; kidney, 40; and blood, 6. This analysis suggests that blood is a poor indicator of the 2,3,7,8-TCDD body burden for

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Table 7.1. Levels of 2,3,7,8-TCDD in soil from different locations

Site	Sample history	TCDD concentration[a,b] (ng/g)	References
Love Canal, NY	Soils outside the dump site	ND (0.001-0.020)	EPA 1985b
Jacksonville, AR	Waste disposal site	ND-2.9	EPA 1985b
Midland, MI	Inside DOW facility	0.01-52	Nestrick et al. 1986
St. Louis, MO	Urban sample of no obvious source of contamination	0.12	EPA 1985b
Shenandoah Stables, MO	Contaminated by waste oil	101-33,000	Tiernan et al. 1985, Kimbrough et al. 1977
Timberline Stables, MO	Contaminated by waste oil	30-42	Tiernan et al. 1985
Bliss Farm, MO	Contaminated by waste oil	382[c]	Tiernan et al. 1985
Bubbling Springs Ranch, MO	Contaminated by waste oil	76-95	Tiernan et al. 1985
Minker Resident, MO	Contaminated by waste oil	50[c]	Tiernan et al. 1985
Times Beach, MO	Contaminated by waste oil	4.4-317	Tiernan et al. 1985
Urban areas, United States	Urban samples of no obvious source of contamination	<0.0002-0.009	Nestrick et al. 1986
New Jersey	Spillage of 2,4,5-TCP still bottom	26,000[c]	Jackson et al. 1986
New Jersey	Scrap yard where used reactor vessels were collected	1,100[c]	Jackson et al. 1986
Lansing, MI	Urban sample	ND(0.0007)-0.003	Nestrick et al. 1986
Gaylord, MS	Urban sample	ND(0.0002)	Nestrick et al. 1986
Detroit, MI	Urban sample	0.0021-0.0036	Nestrick et al. 1986

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Table 7.1 (continued)

Site	Sample history	TCDD concentration[a,b] (ng/g)	References
Chicago, IL	Urban sample	0.0042-0.0094	Nestrick et al. 1986
Akron, OH	Urban sample	0.0063	Nestrick et al. 1986
Nashville, TN	Urban sample	0.0008	Nestrick et al. 1986
Pittsburgh, PA	Urban sample	0.0026	Nestrick et al. 1986
Philadelphia, PA	Urban sample	0.0009	Nestrick et al. 1986
Brooklyn, NY	Urban sample	0.0026	Nestrick et al. 1986
Arlington, VA	Urban sample	ND(0.0003)	Nestrick et al. 1986

[a]ND = not detected.
[b]Values within parentheses are detection limits.
[c]Only one sample was analyzed.

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humans. The levels of 2,3,7,8-TCDD in adipose tissue in the general population of the United States and Canada ranged from undetectable to 20 pg/g, with a mean value of 5 to 7 pg/g. In a U.S. National Human Adipose Tissue Survey, 2,3,7,8-TCDD was detected with a frequency of 76%. In Europe, the range of 2,3,7,8-TCDD concentration in the adipose tissue of the general population is not detectable to 9 pg/g, with a mean value of 3 pg/g. Instances of higher levels in adipose tissue have been reported in individuals exposed to this chemical either during spraying herbicides containing 2,4,5-T or during accidental capacitor or transformer fires. For example, the adipose tissue of a few exposed individuals in the State Office Building fire in Binghamton, New York, had 2,3,7,8-TCDD concentrations in the range 11.6 to 28.3 pg/g, with a mean value of 17.4 pg/g. The adipose tissue of a few heavily exposed individuals involved in spraying operations in Vietnam had 2,3,7,8-TCDD levels ranging from undetectable (detection limit, 3 pg/g) to 99 pg/g, with a mean value of 37 pg/g; however, no difference in the level of 2,3,7,8-TCDD was found in lightly exposed, possibly exposed, and other Vietnam veterans who sought medical help compared with the control population group (EPA 1986a; Gross et al. 1984; Weerasinghe et al. 1986; Patterson et al. 1987c; Nygren et al. 1986; Schecter et al. 1985, 1986; Graham et al. 1986; Stanley et al. 1986; Young 1984). The median concentrations of 2,3,7,8-TCDD (concentration range in parentheses) in control and exposed populations in Missouri have been reported to be 6.4 pg/g (1.4 to 20.2 pg/g) and 17 pg/g (2.8 to 750 pg/g), respectively (Patterson et al. 1986).

Human breast milk has also been analyzed for 2,3,7,8-TCDD. It has been reported that human breast milk is the largest contributor towards the body intake of 2,3,7,8-TCDD in breast-fed babies (Rappe et al. 1986). The following levels (the detection limits are given in parentheses) of 2,3,7,8-TCDD have been determined in human breast milk from different countries: United States, none detected (0.1 to 6.0 pg/g) in mothers from 2,4,5-T-exposed areas and in control areas; Seveso, Italy, 2.3 to 28.0 pg/g from mothers near accident area; South Vietnam, none detected (0.5 pg/g) to 40 to 50 pg/g from mothers in sprayed areas; Sweden, trace to 2.3 pg/g, with a mean of 0.6 pg/g; and Germany, 1.3 to 3.3 pg/g, with a mean of 1.9 pg/g. 2,3,7,8-TCDD was also detected in human milk obtained from Denmark, the Netherlands, and Yugoslavia (Heath et al. 1986, Patterson et al. 1986, Young 1984, Rappe et al. 1986, Jensen 1987, Nygren et al. 1986, EPA 1985b). There is a large unexplainable difference in the values of 2,3,7,8-TCDD concentrations in milk from South Vietnamese mothers analyzed by two groups of investigators. In addition to 2,3,7,8-TCDD, mother's milk has been found to contain 1,2,3,7,8-pentachlorodibenzo-p-dioxin at levels ≥10 ng/g. This congener has rarely been reported as a contaminant in any commercial product other than in commercial pentachlorophenol (Hagenmaier 1986); however, it has always been found in samples from municipal and industrial waste incinerators. Given this finding, a 1-year moratorium for the construction of new municipal incinerators in Sweden was put into effect (Nygren et al. 1986).

Other human tissues, obtained from the autopsy of two subjects in Canada, were analyzed for 2,3,7,8-TCDD with the following results: liver, none detected to 2.5 pg/g; muscle, none detected; and kidney,

none detected. The detection limit in these determinations were in the range of 1 to 4 pg/g (Ryan et al. 1985b). No 2,3,7,8-TCDD was detected in the blood of exposed workers following the accident in Binghamton, New York, at a detection limit of 1 to 2 pg/g (Schecter et al. 1985). Serum 2,3,7,8-TCDD levels in veterans who were heavily exposed to Agent Orange in Vietnam during 1967-1968 have been reported by the Center for Disease Control to range between none detected (0.0013 pg/g) and 25 pg/g, with a median value of 3.8 pg/g. The same study reported serum 2,3,7,8-TCDD levels in a group of non-Vietnam veterans to range between none detected (0.0013 pg/g) and 12 pg/g, with a median value of 3.9 pg/g. Serum levels in a group occupationally exposed to 2,3,7,8-TCDD prior to 1970, however, have been reported to be 30-fold higher in the same study (MMWR, 1987).

7.3 OCCUPATIONAL EXPOSURES

Occupational exposures to 2,3,7,8-TCDD may occur during the production and use of hexachlorophene, trichlorophenol, and herbicides containing 2,4,5-T. The heaviest exposure may occur during the step that is used to purify 2,4,5-T from its contaminants, since these products contain much higher levels of 2,3,7,8-TCDD than the purified products. No data on the occupational exposure to 2,3,7,8-TCDD during the manufacture of these chemicals are available (Rappe 1984). The indirect evidence of occupational exposure to 2,3,7,8-TCDD is the significantly higher adipose tissue levels of the compound in heavily exposed Vietnam veterans and in certain workers at the State Office Building in Binghamton, New York, following the transformer fire.

7.4 POPULATIONS AT HIGH RISK

From the monitoring data discussed in Sects. 7.2 and 7.3, it is possible to predict the segments of the general population and of occupational groups that may be exposed to higher levels of 2,3,7,8-TCDD. Among the occupational groups, workers involved in the production or use of trichlorophenol or its salts, hexachlorophene, and 2,4,5-T or other herbicides containing 2,4,5-T are susceptible to exposure to higher levels of 2,3,7,8-TCDD than the general population. 2,4,5-T and 2,4,5-trichlorophenol and its salts, however, are no longer manufactured in the United States (SRI 1987). Since both flue gases and ashes from municipal and industrial incinerators contain 2,3,7,8-TCDD, workers in this profession are expected to be at higher risk. Populations residing near municipal incinerators may also be subjected to exposure. Workers at sites of improper chemical waste disposal (from trichlorophenol, hexachlorophene, 2,4,5-T, and associated industries) and the general population residing near those sites are potentially exposed to 2,3,7,8-TCDD. Breast-fed babies nursed by mothers residing near improperly controlled municipal incinerators or other sources of exposure are expected to receive 2,3,7,8-TCDD through the milk.

Studies in humans have not demonstrated that there is a sensitive subpopulation. Animal studies, however, suggest that the fetus and newborn infants may represent such a sensitive population. As discussed in Sects. 4.3.3 and 4.3.4 on developmental and reproductive toxicity, 2,3,7,8-TCDD is a demonstrated teratogen in rats and mice, and also results in spontaneous abortions and fetal death in monkeys. Since these

effects occur at low doses, and in the case of teratologic effects at doses that do not appear to adversely affect the mother, it is likely that, at certain stages of fetal development, the fetus represents a sensitive subgroup. Animal data also demonstrate that toxic levels of 2,3,7,8-TCDD can be ingested during nursing and that lactation is a major route for elimination of 2,3,7,8-TCDD. This indicates that the newborn might receive greater exposure than the adult, since no data were found to indicate that newborns are more sensitive than adults.

8. ANALYTICAL METHODS

Several methods are available for the analysis of 2,3,7,8-TCDD in different media. Some of the more recent methods are given in Tables 8.1 and 8.2. The methods listed in these tables are not exhaustive but are illustrative of a few recent methods. Methodologies for collecting samples before their analysis are important, since the concentrations of 2,3,7,8-TCDD in most samples are low. This is particularly important for stack samples that exist both in the vapor and particulate phase. The details of stack-sampling methods are available in Velzy (1986), Ozvacic (1986), and Brenner (1986).

The accuracy of analysis has increased in recent years with the availability of stable isotope-labeled (^{37}Cl and ^{13}C) 2,3,7,8-TCDD for use as an internal standard in mass spectral analysis. With a combination of one of several methods available for sample cleanup, high-performance gas chromatography (GC) (HRGC), and high-resolution mass spectrometry (MS) (HRMS), unequivocal identification and quantification of 2,3,7,8-TCDD can be performed at very low levels. Although negative chemical ionization MS (NCI/MS) shows a higher sensitivity to all other polychlorinated dibenzo-p-dioxins than electron impact MS (EI/MS), it has a lower sensitivity for 2,3,7,8-TCDD (Buser et al. 1985). The analysis of fly ash samples poses a special challenge because of poor solvent extraction recovery and the difficulty in the resolution of 2,3,7,8-TCDD from a large number of congeners present in these samples. Best results were obtained by using digestion with excess dilute HCl, followed by freeze-drying of the residue and hot extraction with toluene. The HCl treatment opens the pore structure of fly ash to permit access to the solvent, and freeze-drying removes water to improve material transfer from the hydrophilic surface of the fly ash to the solvent (Stieglitz et al. 1986). Emphasis has been placed on the analysis of adipose tissue and mother's milk, because these two tissues may be indicators of the human body burden for 2,3,7,8-TCDD. The analysis of fish is also important since some bottom feeders (e.g., channel catfish and carp) and those fish that feed upon bottom feeders may be indicators of 2,3,7,8-TCDD-polluted water (Ryan et al. 1984a, Jensen 1987).

Besides the commonly used analytical methods, other newly developed but yet generally untested methods are available for the analysis of 2,3,7,8-TCDD. Some of these methods are GC with a polymeric liquid crystal capillary column (Naikwadi and Karasek 1986), GC with matrix isolation Fourier transform infrared spectrometry (Wurrey et al. 1986), and HRGC with microwave-induced plasma detection (Mohamad et al. 1986). More detailed descriptions of the analytical methods for 2,3,7,8-TCDD can be found in Buser et al. (1985), Tiernan et al. (1985), Rappe (1984), and EPA (1985b).

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8.1 ENVIRONMENTAL MEDIA

8.1.1 Air, Water, Soil, and Food

See Table 8.1.

8.2 BIOMEDICAL SAMPLES

8.2.1 Fluids/Exudates and Tissues

See Table 8.2.

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Table 8.1. Analytical methods for environmental samples

Sample matrix	Sample preparation	Analytical method[a]	Detection limit	Accuracy[a]	References
Ambient room air following accidental transformer fire	Vapor phase sample collected by silica gel; particulate sample collected on glass fiber filter, solvent extracted, and precleaned by alumina and carbon	HRGC/HRMS	0.003 pg/m³	131 ± 27% at 5-10 pg/m³	Smith et al. 1986
Ambient outdoor air	Vapor and particulate collected on polyurethane foam and glass fiber, precleaned by Florisil and modified silica	HRGC/NICI/MS	0.1-0.2 pg/m³ (method detection limit too high for TCDD determination)	NA	Oehme et al. 1986
	Vapor and particle collected on polyurethane and glass fiber, precleaned by silica and alumina	HRGC/NIEC/MS-SIM	NR (TCDD not separated from other tetra isomers)	NA	Eitzer and Hites 1986
Stack emission	Vapor and particle collected on XAD-2 and glass fiber by modified EPA method 5; solvent extracted and precleaned by silica, alumina, and Biobead	HRGC/MS	NR	NR	Hagenmaier et al. 1986
Fly ash from municipal incinerator	Solvent extraction, HPLC separation on normal-phase and reverse-phase column	GC/MS or HRGC/FID	NR (TCDD not separated from other tetra isomers)	NR	Tong et al. 1984, Tong and Karasek 1986
	Solvent extraction, precleaning by two adsorbent columns, and further fractionation on reverse- and normal-phase HPLC	GC/LRMS	40 pg	NR	Lamparski and Nestrick 1980
	Solvent extraction, precleaned on silica and alumina	HRGC/LRMS	NR	NR	Buser and Rappe 1980
Soil	Solvent extraction, KOH wash, precleaned in alumina, reversed-phase HPLC, and carbon	HRGC/LRMS	3 ng/g	NR	Donnelly et al. 1986
	Solvent extraction, KOH wash, precleaned in chemically treated silica, basic alumina	HRGC/LRMS	NR	NR	Freeman et al. 1986

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Table 8.1 (continued)

Sample matrix	Sample preparation	Analytical method[a]
Water	Sample passed through glass fiber filter and adsorbent cartridge; solvent extracted and precleaned by acid alumina, graphitized carbon and alumina	GC/MS
Chicken and pork	Fat and liver solvent extracted, partitioned with concentrated H_2SO_4, cleaned by Florisil and reverse-phase HPLC	HRGC/MS/MS
Fish	Homogenized fillet digested with ethanolic KOH and solvent extracted; extract cleaned by silica gel-supported H_2SO_4 column and HPLC	HRGC/MS
	Homogenized fillet solvent extracted, partitioned with concentrated H_2SO_4, cleaned by Florisil	HRGC/HRMS
	Homogenate digested with concentrated HCl and solvent extracted; extract cleaned by silica gel-supported H_2SO_4, chemically treated silica and alumina, and reverse-phase HPLC	HRGC/MS/MS
	Digested with alkali, solvent extracted, washed with concentrated H_2SO_4, and cleaned up by site exclusion chromatography, normal- and reverse-phase HPLC	HRGC/EC
Fish and herring gull	Solvent extracted, cleaned up by potassium silicate-silica gel, cesium silicate-silica gel, carbon, and H_2SO_4-silica gel cesium silicate and alumina	HRGC/LRMS

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Detection limit	Accuracy[a]	References
1 pg/L	88% at 6.5 pg/L	O'Keefe et al. 1986
2-4 pg/g	NR	Ryan et al. 1985a
5-10 pg/g	NR	Fehringer et al. 1985
2-10 pg/g	NR	Ryan et al. 1984
<1 pg	NR	Clement et al. 1986
12 pg/g	105% at 18-45 pg/g	Niemann 1986
1-8 pg/g	NR	Stalling et al. 1983, Rappe 1984

Table 8.1 (continued)

Sample matrix	Sample preparation	Analytical method[a]	Detection limit	Accuracy[a]	References
Fish, egg, or sediment	Added HCl and solvent extracted; extract cleaned by gel permeation, trisodium phosphate, H_2SO_4, alumina and carbon columns	HRGC/EC/LRMS	NR	72%	Lawrence et al. 1986

[a]HRGC = High-resolution gas chromatography; NICI/MS = negative ion chemical ionization mass spectrometer; NIEC/MS = negative ion electron impact mass spectrometer; SIM = selective ion monitoring; FID = flame ionization detector; LRMS = low-resolution mass spectrometer; EC = electron capture detector; NR = not reported; NA = not applicable.

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Table 8.2. Analytical methods for biomedical samples

Sample matrix	Sample preparation	Analytical method[a]	Detection limit (pg/g)	Accuracy[a]	References
Human milk	Saponification with hot alkali; solvent extraction, cleaned with concentrated H_2SO_4, alumina	HRGC/HRMS and LRGC/HRMS	0.1 0.6; 0.5-6	NR	Heath et al. 1986
	Extraction with potassium oxalate and mixture of solvents, cleaned by gel permeation, Florisil	HRGC/LRMS	5 (fat basis)	NR	Fuerst et al. 1986
	Acidic digestion, solvent extraction multiple cleanups with adsorbents and chemically modified adsorbents, normal- and reverse-phase HPLC	LRGC/LRMS	0.5	(78 ± 13)% at 1-12 pg/g	Langhorst and Shadoff 1980
Adipose tissue	Tissue solvent extracted and subjected to eight different preparations:		<5	At 50 pg/g:	Albro et al. 1985
	Cleaned through potassium silicate/silica gel, carbon, H_2SO_4/silica and alumina	HRGC/LRMS		70%	
	Washed with concentrated H_2SO_4 and passed through silica, chemically treated silica, alumina, reverse- and normal-phase HPLC	HRGC/LRMS		78%	
	Digested in concentrated HCl and cleaned up by silica/H_2SO_4, alumina, carbon/celite	HRGC/LRMS		92%	
	Saponified with alkali, solvent extracted, and cleaned up by alumina, charcoal/silica	HRGC/LRMS		88%	
	Saponified with alkali, solvent extracted, washed with concentrated H_2SO_4, and chromatographed on silica acid and alumina	HRGC/HRMS		90%	
	Washed with H_2SO_4 and chromatographed on Florisil	HRGC/CIMS		72%	
	Washed with H_2SO_4, chromatographed on alumina, charcoal/celite, alumina	HRGC/HRMS		84%	
	Passed through silica/potassium silicate, silica/carbon, potassium silicate/H_2SO_4/silica and alumina	HRGC/NICI/MS		90%	

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Table 8.2 (continued)

Sample matrix	Sample preparation	Analytical method[a]	Detection limit (pg/g)	Accuracy[a]	References
Adipose tissue	Automated extraction and enrichment apparatus consisting of solvent extraction, cleanup by carbon and silicate/silica gel	HRGC/MS or HRGC/HRMS	<2	>85% at 24 pg/g	Lapeza et al. 1986, Patterson et al. 1987a
Blood, liver, kidney and muscle	Homogenized sample solvent extracted, cleaned up with H_2SO_4, chromatography, or Florisil	HRGC/MS/MS	1–4	NR	Ryan et al. 1985b,c
Serum	Solvent extraction, concentrated sulfuric acid wash and cleanup by carbon and silicate/silica gel, followed by silicate/H_2SO_4/silica gel and alumina columns	Isotopic dilution with HRGC/HRMS	1.25×10^{-3}	89% at 5.0 μg/mL	Patterson et al. 1987b

[a]HRGC = High-resolution gas chromatography; NICI/MS = negative ion chemical ionization mass spectrometer; HRMS = high-resolution mass spectrometry; MS = mass spectrometry; LRMS = low-resolution mass spectrometer; CIMS = chemical ionization mass spectrometry; NR = not reported.

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9. REGULATORY AND ADVISORY STATUS

9.1 INTERNATIONAL (WORLD HEALTH ORGANIZATION)

No World Health Organization standards were found. The World Health Organization advisory developed by IARC is presented below.

9.2 NATIONAL

9.2.1 Regulations

The reportable quantity (RQ) for 2,3,7,8-TCDD is 1 lb, which places 2,3,7,8-TCDD in category X. The authority for listing 2,3,7,8-TCDD as a CERCLA hazardous substance is provided by Section 307(a) of the Clean Water Act (FR 50, No. 65, p. 13456).

9.2.2 Advisory Guidance

Air. No health advisories (HAs) for levels of 2,3,7,8-TCDD in air were encountered.

Water.

AGENCY	ADVISORY
EPA	Drinking water advisories: 1-day HA--1.0×10^{-6} mg/L (child) 10-day HA--1.0×10^{-7} mg/L (child) Long-term HA--1.0×10^{-8} mg/L (child) Long-term HA--3.5×10^{-8} mg/L (adult) Drinking water equivalent level--3.5×10^{-8} mg/L 10^{-4} to 10^{-7} excess cancer risk--2.2×10^{-8} to 2.2×10^{-11} mg/L (EPA 1986b)
EPA	Ambient water quality criteria 10^{-4} to 10^{-7} excess cancer risk--1.3×10^{-9} to 1.3×10^{-12} mg/L (EPA 1984)

Food.

AGENCY	ADVISORY
FDA	Levels in fish: No serious health concerns--<25 ppt (EPA 1985b)

9.2.3 Data Analysis

Reference doses (RfDs). EPA (1985a) calculated a chronic oral RfD for 2,3,7,8-TCDD based on the data from a three-generation study in rats by Murray et al. (1979), as reanalyzed by Nisbet and Paxton (1982). In this study, rats were exposed to diets containing 2,3,7,8-TCDD at levels

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that provided doses of 0.001, 0.01, and 0.1 μg/kg/day. The highest dose resulted in decreased fetal survival; the middle dose resulted in effects on litter size and fetal and neonatal survival. The lowest dose resulted in dilated renal pelvises, decreased fetal weight, and changes in the gestational index. Therefore, the dose of 0.001 μg/kg/day is a LOAEL, and the RfD was calculated as follows:

$$RfD = (0.001\ \mu g/kg/day)/(100)(10) = 0.000001\ \mu g/kg/day\ ,$$

where: 0.001 μg/kg/day = LOAEL,
100 = uncertainty factor for inter- and intraspecies extrapolation,
10 = uncertainty factor for use of a LOAEL.

Carcinogenic potency, q_1*. EPA (1985b) has classified 2,3,7,8-TCDD in CAG Group B2 when 2,3,7,8-TCDD is considered alone, and in Group B1 when 2,3,7,8-TCDD is considered in association with phenoxyherbicides and/or chlorophenols. Group B2 indicates that although evidence in humans is inadequate, there are sufficient animal carcinogenicity data to consider 2,3,7,8-TCDD a probable human carcinogen. Group B1 indicates that there are not only sufficient animal data but also limited human data to support the consideration that 2,3,7,8-TCDD, in conjunction with phenoxyherbicides and/or chlorophenols, is a human carcinogen. IARC (1982) has classified 2,3,7,8-TCDD in Group 2B, which is analogous to the CAG classification of Group B2. NIOSH (1984) recommended that 2,3,7,8-TCDD be considered a potential occupational carcinogen and that exposure be limited to the fullest extent feasible.

EPA (1985a) developed a quantitative unit cancer risk estimate based on the study by Kociba et al. (1978a,b) and a reexamination of the histologic evidence from that study conducted by Squire for the EPA. The calculations were based on the increased incidence of tumors of the lungs, liver, hard palate, and nasal turbinates in rats maintained on diets containing 2,3,7,8-TCDD for 2 years. The two different pathologic examinations produced differences in tumor incidence and, hence, different q_1* values. The final value derived, $1.56 \times 105\ (mg/kg/day)^{-1}$, was an average of the values for each pathologic evaluation.

Carcinogenic potency, methods used by other agencies. Both the Center for Disease Control (CDC) and the Food and Drug Administration (FDA) have calculated a virtual safe dose for 2,3,7,8-TCDD, which corresponds to an excess cancer risk of 10^{-6} (Hiremath et al. 1986, Kimbrough et al. 1984). The FDA calculations were based on the Kociba et al. (1978a,b) study, whereas the CDC calculations were based on the Squire evaluation of the Kociba et al. (1978a,b) study. Thus, human intake values for a virtual safe dose derived by EPA, CDC, and FDA, respectively, are 6.4, 27.6, and 57.2 fg/kg/day.

9.3 STATE

Minnesota, Michigan, and New York have set additional regulations on the allowable levels of 2,3,7,8-TCDD. In addition, other states may either have regulations or are in the process of promulgating regulations. (Regulations and advisory guidance from the states were still being compiled at the time of printing.)

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11. GLOSSARY

Acute Exposure--Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Bioconcentration Factor (BCF)--The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same time period.

Carcinogen--A chemical capable of inducing cancer.

Ceiling value (CL)--A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure--Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity--The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Embryotoxicity and Fetotoxicity--Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

Frank Effect Level (FEL)--That level of exposure which produces a statistically or biologically significant increase in frequency or severity of unmistakable adverse effects, such as irreversible functional impairment or mortality, in an exposed population when compared with its appropriate control.

EPA Health Advisory--An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)--The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects.

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Intermediate Exposure--Exposure to a chemical for a duration of 15-364 days, as specified in the Toxicological Profiles.

Immunologic Toxicity--The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

In vitro--Isolated from the living organism and artificially maintained, as in a test tube.

In vivo--Occurring within the living organism.

Key Study--An animal or human toxicological study that best illustrates the nature of the adverse effects produced and the doses associated with those effects.

Lethal Concentration(LO) (LC_{LO})--The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration(50) (LC_{50})--A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose(LO) (LD_{LO})--The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

Lethal Dose(50) (LD_{50})--The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)--The lowest dose of chemical in a study or group of studies which produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lowest-Observed-Effect Level (LOEL)--The lowest dose of chemical in a study or group of studies which produces statistically or biologically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

Malformations--Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level--An estimate of daily human exposure to a chemical that is likely to be without an appreciable risk of deleterious effects (noncancerous) over a specified duration of exposure.

Mutagen--A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

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Neurotoxicity--The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)--That dose of chemical at which there are no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

No-Observed-Effect Level (NOEL)--That dose of chemical at which there are no statistically or biologically significant increases in frequency or severity of effects seen between the exposed population and its appropriate control.

Permissible Exposure Limit (PEL)--An allowable exposure level in workplace air averaged over an 8-h shift.

q_1^*--The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1^* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually $\mu g/L$ for water, mg/kg/day for food, and $\mu g/m^3$ for air).

Reference Dose (RfD)--An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)--The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are: (1) 1 lb or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-h period.

Reproductive Toxicity--The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Short-Term Exposure Limit (STEL)--The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity--This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen--A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)--A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-weighted Average (TWA)--An allowable exposure concentration averaged over a normal 8-h workday or 40-h workweek.

Uncertainty Factor (UF)--A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of humans, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIXES

D-5137

APPENDIX A: PEER REVIEW

A peer review panel was assembled for 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). The panel consisted of the following members: Dr. Herbert Cornish, University of Michigan; Dr. Shane Que Hee, University of Cincinnati Medical Center; and Dr. James Olson, State University of New York at Buffalo, School of Medicine. These experts collectively have knowledge of 2,3,7,8-TCDD's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in the Superfund Amendments and Reauthorization Act of 1986, Section 110.

A joint panel of scientists from ATSDR and EPA has reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated into the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in this record.

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APPENDIX B: FEDERAL REGISTER ANNOUNCEMENT

DEPARTMENT OF HEALTH AND HUMAN SERVICES
AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY
ENVIRONMENTAL PROTECTION AGENCY
(ATSDR-2; FRL-3269-7)

NOTICE OF AVAILABILITY OF TOXICOLOGICAL PROFILES

AGENCIES: Department of Health and Human Services (DHHS): Agency for Toxic Substances and Disease Registry (ATSDR); and Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: The Superfund Amendments and Reauthorization Act (SARA) (Public Law 99-499) amends the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA or Superfund) (42 U.S.C. 9601 et seq.) by establishing certain requirements for the Agency for Toxic Substances and Disease Registry (ATSDR) of DHHS and EPA with regard to hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List (NPL). Among these statutory requirements is a mandate for the Administrator of ATSDR to prepare toxicological profiles for each substance previously included on the first priority list of 100 chemicals. The list identified the first 100 chemicals which both Agencies determined posed the most significant potential threat to human health. This list was published in the Federal Register on April 17th, 1987 (52 FR 12866) as required by SARA section 110.

This notice announces the expected availability dates of the first 25 draft toxicological profiles for review and comment.

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AVAILABILITY: The following draft toxicological profiles are expected to be publicly available by the date indicated:

Date/Profile	CAS #
October 17, 1987:	
Benzo(a)anthracene	56-55-3
Benzo(a)pyrene	50-32-8
Beryllium	7440-41-7
Chloroform	67-66-3
Chromium	7440-47-3
Chrysene	218-01-9
Dibenzo(a,h)anthracene	53-70-3
Heptachlor/Heptachlor epoxide	76-44-8 / 1024-57-3
Nickel	7440-02-0
N-Nitrosodiphenylamine	86-30-6
October 29, 1987:	
Aldrin/dieldrin	309-00-2 / 60-57-1
Arsenic	7440-38-2
Benzo(b)fluoranthene	205-99-2
PCBs - Aroclor 1260, 1254, 1248,	11096-82-5, 11097-69-1, 12672-29-6
1242, 1232, 1221,	53469-21-9, 11141-16-5, 11104-28-2
1016	12674-11-2
2,3,7,8- Tetrachlorodibenzo-p-dioxin	1746-01-6
November 5, 1987	
Benzene	71-43-2
Bis(2-ethylhexyl)phthalate	117-81-7
Cadmium	7440-43-9
1,4-Dichlorobenzene	106-46-7
Methylene chloride	75-09-2
November 30, 1987	
Cyanide	57-12-5
Lead	7439-92-1
Tetrachloroethylene	127-18-4
Trichloroethylene	79-01-6
Vinyl chloride	75-01-4

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A full 90-day public comment period will be provided for each profile, starting from the actual release date. The close of the comment period for each draft profile will be indicated on the front of each profile.

Requests for draft toxicological profiles should be sent to:

Ms. Georgi Jones
Director, Office of External Affairs
Agency for Toxic Substances and Disease Registry
Chamblee 28 South
1600 Clifton Rd.
Atlanta, GA 30333

Specify the profiles you wish to review. One copy of each profile requested will be forwarded, free of charge, as they become available. In the case of undue delays, requestors will be notified.

Five copies of all comments should be sent to Ms. Jones at the above address by the end of the comment period. All written comments and the draft profiles will be available for public inspection at the Agency for Toxic Substances and Disease Registry (ATSDR), Building 28 South, Room 1103, 4770 Buford Highway, NE, Chamblee, GA, from 8am to 4:30pm, Monday through Friday, except legal holidays. Written comments and other data submitted in response to this notice and the draft toxicological profiles should bear the docket control number ATSDR-2.

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SUPPLEMENTARY INFORMATION:

I. BACKGROUND

On October 17, 1986, the President signed the Superfund Amendments and Reauthorization Act of 1986 (Public Law 99-499), which extends and amends the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund, 42 U.S.C. 9601 et seq.).

Section 110 of SARA amends section 104(i) of CERCLA by establishing requirements for the preparation of: (1) lists of hazardous substances in order of priority, (2) toxicological profiles of those substances, and (3) a research program to fill data gaps associated with the substances.

In compliance with section 104(i)(2)(A) of CERCLA, ATSDR and EPA published on April 17, 1987 (52 FR 12866) the first priority list of 100 hazardous substances. This priority list of 100 was further broken down into four groups of 25 chemicals. The first group of 25 was to be the subject of the second phase of the requirements, i.e., the development of the first set of toxicological profiles. Section 104(i)(3) of CERCLA spells out the content of these profiles and the timetable by which they must be developed. Profiles on at least 25 substances on the first priority list were to be completed within one year of the enactment of SARA (by October 17, 1987). The remaining seventy-five are to be completed at a rate of at least twenty-five per year with the total 100 completed within four years after the enactment of the SARA amendments. Revision and republication is mandated as necessary but no less often than once every three years.

Each profile is required to include an examination, summary and interpretation of available toxicological information and epidemiologic evaluations. This information and data are to be used to ascertain the levels of significant human exposure for the substance and the associated health effects. The profiles must also include a determination of whether adequate information on the health effects of each substance is available or in the process of development. The Agencies' intention is that this information be used to identify the key toxicological testing needs that when filled will improve our ability to define significant human exposure levels.

The toxicological profiles are to be provided to the States and made available to the public. The profiles are to be prepared in accordance with the guidelines developed by ATSDR and EPA. These guidelines were published along with the priority list of 100 in the April 17, 1987 Federal Register Notice (52 FR 12870).

This current notice announces the projected availability dates of the first 25 draft toxicological profiles. The documents have undergone extensive internal review and have been subject to scientific and technical peer review by outside experts. We are now announcing their availability and encouraging public participation and comment on the further development of these profiles. Although the profiles will not be completed by the October 17, 1987 deadline, we believe that the extra time given to peer review and public review and comment is important to the development of quality profiles of scientific merit.

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Although we are reasonably confident that the key studies for each of the 25 substances were considered during the profile development process, this *Federal Register* notice solicits any significant studies, including unpublished data, which may aid the revision of these draft profiles.

II. LEVELS OF SIGNIFICANT HUMAN EXPOSURE

The setting of specific levels of significant human exposure has presented a unique set of problems. The significance of a specific level of a hazardous substance depends on the context in which that level is evaluated. For example, a low level that may be insignificant with respect to causing acute, immediately debilitating symptoms may be highly significant with respect to causing gradual, chronic effects over a longer term. Since these profiles are intended for use by a diverse group of people who have different situations in which to interpret the significance of specific levels, it was considered appropriate at this time to describe the range of exposures over which effects may occur (where data are available), and to allow the user to make determinations as to which type of effect is significant in any particular instance. A format for graphically displaying the levels of significant human exposure has been developed and is used in the profiles to present the ranges over which effects may be observed.

We encourage public comment and recommendations on this specific issue.

III. SOLICITATION OF PUBLIC COMMENT

We are soliciting public comment on all phases of the development of the toxicological profiles. A previous Federal Register notice, published on April 17, 1987 (52 FR 12866) solicited comment on the first priority list of hazardous substances. We are currently reviewing those comments and are evaluating the impact that those comments may have on the priority list and the methods used in its development.

As the first 25 toxicological profiles become available in draft form, we are eager to provide them to the States, industry, public health professionals, scientists and the general public. We welcome comment and feedback on the content of the profiles; the format and scope of the documents; the process used in the development of the levels of significant human exposure and the overall process used in the development of the profiles.

There are specific items that we would like to draw to the attention of the reader and would strongly encourage as candidates for close attention during the comment period.

A. PUBLIC HEALTH STATEMENT

The draft profiles include a public health statement which is intended to provide the lay public with a concise statement of the general health risks associated with the chemical of concern. The summary as originally planned should be able to stand alone. If removed from the rest of the document, it should still be capable of conveying

to the public the substantive health concerns associated with the substance. We are also considering the development of more abbreviated versions of the public health statements and are evaluating a number of different formats. This notice specifically invites comments on the existing public health effects statements in the draft profiles and solicits recommendations for alternative approaches.

B. DATA/STUDIES USED IN THE DEVELOPMENT OF THE PROFILES

In general, and for each chemical-specific profile, have the appropriate studies been used in the development of these documents? Our concern here is that we capture the critical, or "key", studies but not miss other data that may be important in the valid evaluation of the toxicological profile chemicals.

C. FORMAT AND CONTENT OF THE PROFILES

The draft profiles represent our best effort to provide the information required by Section 104 (i)(3) of CERCLA in the most useful format for the various identified users of the profiles, given the constraints of the tight timeframe. Every effort has been made to define sections clearly and to format the documents in such a way that they can be used as resource documents by many different audiences. We specifically request comment on the format and content of the initial set of profiles, including how the format might be modified for subsequent sets of profiles.

D. LEVELS OF SIGNIFICANT HUMAN EXPOSURE

What is the most useful way of presenting this type of information? For this first generation of profiles we have selected a graphic presentation that reflects a "range" of values that covers both upper and lower bounds of effect levels. Is this more useful than a single number? Are there other ways of presenting this type of information that would be more useful to the eventual user?

E. IDENTIFICATION OF SIGNIFICANT DATA GAPS

The process used to develop the draft profiles has resulted in the identification of the full range of health effects data gaps associated with each chemical. However, depending on individual circumstances some subset of the identified data gaps may be essential in determining levels of significant exposure, while other data gaps may be less immediate. ATSDR, EPA, and the National Toxicology Program (NTP) have been exploring ways to identify the critical data elements that are needed to establish significant human exposure levels. This notice specifically requests comment and suggestions for approaching this phase of the toxicological profile process.

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DEPARTMENT OF
HEALTH & HUMAN SERVICES

Public Health Service
Agency for Toxic Substances and Disease Registry
Atlanta, GA 30333

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DAVIS v. DOW

Exhibits 1 - 50

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DAVIS v. DOW

EXHIBITS 1-277

Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

File T23.14-11-1
Chg. 1219A
Rec'd 9-27-44
Fin'd 10-19-44
Work By H. A. Wyse

Subject

PRELIMINARY INFORMATION RELATIVE TO THE TOXICITY OF 2,4-DICHLOROPHENOXY-ACETIC ACID (K2372-3)

Check Edwin E. Munn Rept. By V. K. Rowe 10-23-44

To Technical Service & Development Div.
(W. W. Allen)
W. R. Veazey
Organic Research Laboratory

Preliminary tests have indicated that 2,4-dichlorophenoxyacetic acid has a moderate acute oral toxicity and that it does not produce marked skin irritation.

We would not expect serious difficulties to result from handling this material in a reasonable manner.

Similar tests will be made on 2,4,5-trichlorophenoxyacetic acid when a sample is available.

1a-4 copies

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[illegible] during a [illegible] period [illegible] effect and Mr. Kephart advised that [illegible] [illegible] Mr. Evans put [illegible] [illegible] suffered any ill [illegible] ingestion. [illegible] [illegible] to completion by these two scientists to ascertain that [illegible] not to be feared for noxious effects.

Mr. Kephart and Mr. Mitchell [illegible] an experiment carried to completion by them in which they [illegible] heavy applications of 2-4-D to certain pasture and [illegible] sheep and goats to browse in the places where [illegible] had been applied. [illegible] animals showed no difference in their liking or dislike of feeding in the places where the formulas had been applied or where there had been no application. Neither did they go to sleep over the effects of any opiate nor was it possible to observe any effect as a result of having eaten the aforementioned chemical.

"They also advised of a case in which a cow was fed daily over a long period of time with greens which contained relatively heavy doses of pure 2-4-D. They pointed out that they were unable to observe any effect of 2-4-D in the cow nor in the calf which was born during the period in which the experiments were carried to completion. They found traces of 2-4-D in the blood of the cow which would indicate that its organism was normally eliminating the chemical product. After some time, the cow was slaughtered and several experts in the animal industry conducted an autopsy analyzing carefully all of the organs of the cow without being able to find any trace of the chemical compound or without perceiving any effect of the 2-4-D.

"Mr. Kephart advised that the manufacturers of 2-4-D had encountered no difficulty in their factories with any of their employees. Those who are employed are in continual contact with the compound in concentrated forms. For this reason, they could not understand why it should be that men who are employed in agricultural fields applying the chemical substances in a much diluted form should suffer any effects adverse to their health. The manufacturers reported on a case in which a man in the State of California working intensively with 2-4-D had broken out in an eruption of the skin but they attributed this case to other chemicals and not to 2-4-D. They said that the eruption disappeared before it was possible to make any study of it with the purpose of determining its cause."

Translated from Spanish
by Joseph E. McKinney
May 7, 1946

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January 3, 1951

W. J. Allen, Sales Dept.
J. D. Kraft, Biochem. Res. Lab.
J. P. [illegible], Coal Branch
G. C. Egan, Agricultural Lab.
[illegible], Legal Dept.

Dr. Willard of Ohio State University presented [illegible] paper at the recent meeting of the [illegible] Weed Control Conference. The title was [illegible] of Herbicides". It will be published [illegible] the Conference proceedings, but I have [illegible] Dr. Willard and will supply it to those indicated above.

Very truly yours,

W. C. Dalton

WCD/ass

bc

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Harrisson, Jos. W. E., and Rees, Edward W. 2,4-D Toxicity-I. Toxicity Toward Certain Species of Fish. American Journal of Pharmacy 118, 422-425 (1946).

The LD_{50} concentration of 2,4-D for minnows is approximately 2,000 p.p.m.; for sunfish, 1,000 p.p.m.; and for catfish, 2,000 p.p.m. The upper safe limit for minnows is 1,500 p.p.m.; sunfish, 500 p.p.m.; and estimated to be about 500 p.p.m. for catfish. These figures refer to a seven-day exposure period during which the concentration of 2,4-D was kept at a fixed level.

(R.H. Summary)

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3. Toxicity of 2,4-Dichlorophenoxyacetic Acid For Experimental Animals. Edwin V. Hill and Harold Carlisle. Journal of Industrial Hygiene and Toxicology. Vol. 29 pp. 85-95. March 1947.

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TOXICITY OF 2,4 DICHLOROPHENOXYACETIC ACID FOR EXPERIMENTAL ANIMALS*

EDWIN V. HILL[1] AND HAROLD CARLISLE[2]

WITHIN the past few months several articles (1) have appeared in the scientific press describing the action and use of 2,4-dichlorophenoxyacetic (2,4-D) acid and its ammonium or sodium salts as herbicides and plant hormones. This compound has also been used in orchards to prevent the premature dropping of apples.

2,4-Dichlorophenoxyacetic acid is prepared commercially by reacting chloracetic acid, 2,4-dichlorophenol, and aqueous alkali or by the direct chlorination of phenoxyacetic acid.

It can be applied to plants either as an aqueous solution or dissolved in tributylphosphate diluted with diesel oil. Potential health hazards may exist through inhalation when disseminated in aqueous or tributyl phosphate-diesel oil mists or by eating leafy vegetables or fruits contaminated by traces of the compound.

Practically no data have been presented in the literature on the toxicity of this and related compounds. Experiments were performed to determine the toxicity of the free acid and its salts for experimental animals when administered orally, parenterally, and by inhalation.

METHODS AND MATERIALS

Commercial 2,4-D contains small amounts of phenolic impurities and related isomeric phenoxyacetic acids. The following procedure was used to remove such impurities as well as inorganic contaminants and traces of colored material.

a. Two hundred and twenty-one grams of 2,4-D were dissolved in 2 liters of 98–100 per cent ethanol with the aid of mild heating to increase rate of solution.
b. The resulting solution was suction filtered to remove small amounts of suspended matter which occasionally were present.
c. The filtered alcoholic solution was saturated with gaseous ammonia until a test portion of the clear supernatant liquid gave no further precipitate of the ammonium 2,4-dichlorophenoxyacetate. The solution was stirred continuously during the addition of the ammonia.
d. The precipitated ammonium salt was suction filtered, dissolved in 2 liters of hot distilled water (about 70° C.), and 10 grams of decolorizing char added to the ammoniacal solution, which was then allowed to stand at 70° C. for one-half hour.
e. The decolorizing char was removed by gravity filtration and the filtrate cooled at 10° C. The ammonium 2,4-dichlorophenoxyacetate gradually crystallized in fine needles, the crystallization being complete after twelve hours.
f. The ammonium salt was suction filtered, dissolved in 2 liters of hot water (70° C.) and the 2,4-dichlorophenoxyacetic acid precipitated by the addition of the alkaline solution, with stirring and cooling, to 150 ml. of concentrated hydrochloric acid (38 per cent S.G. 1.19).
g. The 2,4-dichlorophenoxyacetic acid was suction filtered, washed with distilled water until a test portion of the filtrate gave no precipitate or cloudiness with aqueous silver nitrate, and recrystallized three times from hot water.
h. The filtered product, vacuum dried over concentrated sulfuric acid for seven days, consists of fine transparent needlelike crystals, m.p. 139° C. (A Fisher-Johns melting point apparatus was used for the determination), reported m.p. (literature) 138° C., 139–140° C. The neutralization equivalent was determined for 3 samples, 1) 221.1, 2) 221.1, 3) 221.0.

Toxicity determinations were made on crude and purified 2,4-D. Sodium and ammonium salts of the purified acid were prepared and also assayed for toxicity at concentrations of 1 or 4 per cent. Solutions of the acid or salt were prepared in physiological saline and adjusted to a final pH of 7.0 to 7.2. In the parenteral toxicity tests on these four materials, physiological saline

* Received for publication August 11, 1946. This work was conducted at Camp Detrick, Frederick, Maryland, from December, 1944 to June, 1945.

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was ordinarily used as a volume control but in later experiments a 1.81 per cent sodium chloride solution was used as an osmotic pressure and sodium ion control.

The preparation of oil solutions of crude acid required the use of an intermediate solvent. The acid was first dissolved in either tributyl phosphate or n-butyl alcohol and these solutions were then mixed with the proper amount of diesel oil. Various combinations of 2,4-D, solvent and #2 diesel oil were tested. Each combination tested will be described in detail as the experimental work on the material is presented.

All injections were made by accepted technics. Forced feedings were carried out in the smaller animals by means of a #4 or #5 woven cloth ureteral catheter and in the larger animals by means of a #8 rubber ureteral catheter. The catheters were attached to syringes of appropriate size and were lubricated slightly with petrolatum before use. It was found advantageous to anesthetize white rats and guinea pigs before feeding was carried out. Since nothing was known about the length of time necessary for effects to occur, all animals were held for more than an adequate number of days during the first part of the work. It was found that deaths seldom occurred after the fifth day so that in the later experiments animals were sacrificed on the sixth or seventh day.

Results

The Acute Toxicity of the Purified Sodium Salt of 2,4-D

A total of 17 experiments was carried out to determine the toxicity of the purified sodium salt of 2,4-D for the various experimental animals when given by injection or feeding with a stomach tube. In these experiments approximately 450 mice, 150 white rats, 125 guinea pigs, 70 rabbits and 3 monkeys were used. Since the titrations proved to be more precise and repeatable than titrations involving living agents such as bacteria, it was found possible to arrive at a fairly accurate LD_{50} by using only 10 mice, 6 rats, 6 guinea pigs, and 4 rabbits for each 4 or 5 dilutions, covering a comparatively small range of concentrations. For example, the mouse titrations consisted of the injection or feeding of 2.5 mg., 5.0 mg., 7.5 mg., and 10 mg. of 2,4-D. The amounts usually administered to guinea pigs were 100 mg., 150 mg., 200 mg., 250 mg., and 300 mg. Most of the titrations were carried out at least twice and in every case replicates agreed satisfactorily. The toxic and tolerated doses determined by these 17 titrations are given in Table 1. The LD_{50} of 2,4-D injected intravenously in rabbits is approximately 400 mg./kg. About 50 per cent of the animals die suddenly of acute ventricular fibrillation and those that survive this acute episode die within several hours to three days. The outstanding symptoms were referable to the skeletal muscle where a condition not unlike myotonia congentia was present. The animals received symptomatic relief by the administration of quinine hydrochloride, but a fatal outcome could not be avoided.

The symptoms observed in the smaller animals were, in order of appearance, stiffness of the extremities with some muscular incoordination, lethargy, paralysis of the hindquarters, stupor, coma, and finally, death. These symptoms were observed consistently regardless of the method of administration of the chemical.

The data indicated that the toxic and tolerated doses are slightly higher when the material is given by stomach tube. As would be expected, animals fed with 2,4-D did not develop symptoms as early as did injected animals. In addition, symptoms were more variable in degree in animals fed by stomach tube and the final results of such titrations were less precise and repeatable. Table 2 summarizes the results of one of several experiments carried out in which comparative data on toxicity by feeding and injection were obtained.

Three monkeys were fed varying amounts of the material. One monkey receiving 0.75 gm. exhibited only slight atypical symptoms and recovered completely. Two additional monkeys were fed 1.0 gm. and 1.5 gm. each, but both animals became ill about four hours after being fed and regurgitated a large portion of the material so that no information on lethal doses for monkeys by feeding is available. No typical symptoms appeared in any of the monkeys, and all were apparently normal seven days after being fed. One animal, which was fed 1.0 gm. of 2,4-D, was kept under observation for an additional sixty days without the appearance of any delayed effects. Two of these same monkeys were then used in injection experiments. The monkey which had been fed 0.75 gm. was injected intraperitoneally with 1.0 gm. of the purified sodium salt of 2,4-D. This animal developed nausea and vomiting,

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stiffness of the legs with some muscular incoordination, lethargy, hanging of the head and ptosis of the eyelids. The symptoms appeared about two hours after inoculation, persisted for about forty-eight hours, and then began to lessen in intensity. At the end of five days, the animal had recovered and was apparently normal. The second monkey, which had been fed 1.5 gm. seven days previously, was injected intraperitoneally with 1.5 gm. of the same material. This animal showed symptoms similar to those seen in the first injected monkey and by five days had recovered completely.

TABLE 1

TOXIC AND TOLERATED DOSES OF THE SODIUM SALT OF 2,4-D FOR EXPERIMENTAL ANIMALS BY INJECTION AND FEEDING

SPECIES	METHOD OF ADMINISTRATION	APPROXIMATE WEIGHT OF ANIMAL	TOXIC DOSE*		TOLERATED DOSE**	
			Per Animal	mg./kg.	Per Animal	mg./kg.
White mouse	Intraperitoneal	20 gm.	7.5 mg.	375	2.5 mg.	125
White mouse	Stomach tube	20 gm.	7.5 mg.	375	2.5 mg.	125
Guinea pig	Intraperitoneal	300 gm.	200 mg.	666	100 mg.	333
Guinea pig	Stomach tube	300 gm.	300 mg.	1000	100 mg.	333
Rabbit	Intraperitoneal	2.5 kg.	1.0 gm.	400	500 mg.	200
Rabbit	Stomach tube	2.5 kg.	2.0 gm.	800	500 mg.	200
Rabbit	Intravenous	2.5 kg.	1.0 gm.	400	500 mg.	200
White rat	Intraperitoneal	150 gm.	100 mg.	666	25 mg.	166
White rat	Stomach tube	150 gm.	100 mg.	666	50 mg.	333
Monkey	Intraperitoneal	3.5 kg.			1.5 gm.	428
Monkey	Stomach tube	3.5 kg.			750 mg.	214

* Toxic dose—50 per cent mortality.

** Tolerated dose—largest amount causing no deaths.

TABLE 2

COMPARISON OF THE TOXICITY OF THE PURIFIED SODIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID FOR WHITE RATS BY INJECTION AND FEEDING

MG 2,4-D GIVEN	ROUTE	APPEARANCE OF SYMPTOMS	MORTALITY*
50	I.P.	1 hour	2/6
100	I.P.	30 min.	6/6
150	I.P.	30 min.	6/6
200	I.P.	30 min.	6/6
50	Feed	4–6 hours	0/6
100	Feed	2–3 hours	4/6
150	Feed	1–2 hours	5/6
200	Feed	1–2 hours	6/6
Saline control	I.P.	None	0/6
Saline control	Feed	None	0/6

* Mortality data recorded as ratio of deaths to total animals used. Rats used in this experiment averaged 150 gm. in weight.

Toxicity of Crude Versus Purified 2,4-D

Five titrations were carried out to compare this toxicity of purified sodium salt of 2,4-D with that of the crude material as it comes from the manufacturer. In these experiments, the crude acid was first converted to the sodium salt and was used in this form with all impurities present. The results, as presented in Table 3, indicate that there was no significant difference in the toxicity of the two materials either by injection or feeding.

The Toxicity of Pure 2,4-D

Two experiments were carried out to test the toxicity of pure 2,4-D. Because of its insolubility, the material was suspended in 5 per cent gelatin solution and fed to white rats by means of a stomach tube. The addition of gelatin to the suspension prevented settling and agglomeration of the particles. No injection experiments were carried out, but the results of the two feeding titrations summarized in Table 4 indicated that 2,4-D possesses about the same toxicity as does its sodium salt.

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TABLE 3

A COMPARISON OF THE TOXICITY OF PURIFIED AND CRUDE SODIUM SALT OF 2,4-D BY INJECTION AND BY FEEDING

SPECIES	MG./KG.	ROUTE	MORTALITY***		
			Purified	Crude	Controls
White mouse	500	I.P.*	10/10	10/10	
	375	I.P.	3/10	4/10	
	250	I.P.	0/10	0/10	
	125	I.P.	0/10	0/10	
	Saline control	I.P.			0/10
White rat	1000	I.P.	5/6	6/6	
	666	I.P.	5/6	4/6	
	333	I.P.	0/6	0/6	
	167	I.P.	0/6	0/6	
	Saline control	I.P.			0/6
Guinea pig	833	I.P.	6/6	4/6	
	667	I.P.	5/6	4/6	
	500	I.P.	1/6	2/6	
	33	I.P.	0/6	0/6	
	Saline control	I.P.			0/6
Rabbit	400	I.P.	3/3	1/3	
	200	I.P.	0/3	0/3	
	Saline control	I.P.			0/3
White rat	1000	Feed**	5/6	5/6	
	666	Feed	3/6	2/6	
	333	Feed	1/6	1/6	
	Saline control	Feed			0/6

* I.P.—Intraperitoneal injection of 2,4-D salts in physiological saline solution.
** Feed—Feeding by stomach tube of 2,4-D salts in physiological saline solution.
*** Mortality data are recorded as ratio of deaths to total animals used.

TABLE 4

A COMPARISON OF THE TOXICITY OF PURE 2,4-D AND THE PURIFIED SODIUM SALT OF 2,4-D BY FEEDING TO WHITE RATS AND GUINEA PIGS

SPECIES	MG./KG.	MORTALITY*		
		2,4-D	Sodium Salt of 2,4-D	Controls
White rat	1000	4/4	5/6	
	666	3/4	3/6	
	333	0/4	0/6	
	Gelatin control			0/4
	Saline control			0/6
Guinea pig	33	0/8	0/8	
	Gelatin control			0/4
	Saline control			0/4

* Mortality data recorded as ratio of deaths to total animals used.

The Toxicity of the Ammonium Salt of 2,4-D

Experiments were carried out to compare the toxicity of the ammonium salt of 2,4-D with that of the sodium salt. Amounts of the ammonium salt equivalent to the tolerated dose of the sodium salt for each species were fed to groups of 6 guinea pigs, 6 white rats, and 4 rabbits. In this experiment all animals survived, indicating that the ammonium salt is at least no more toxic than the sodium salt by feeding. Two injection titrations were carried out on white mice and the results indicated that the ammonium and sodium salts possess about the same degree of toxicity by injection also. The preceding two types of experiments were carried out with both the purified salt and the crude salt and no difference in toxicity was detected. One monkey was fed 1.0 gm. of the pure ammonium salt of 2,4-D. The monkey regurgitated a large amount of the material about

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three hours later and was apparently normal when observed the next day. No symptoms other than the nausea were noticed. The results of the experiments on the ammonium salt of 2,4-D are outlined in Table 5.

The Toxicity of Crude Acid 2,4-D Dissolved in either Tributylphosphate or n-Butyl Alcohol and Oil

The toxicity of crude acid 2,4-D dissolved in tributylphosphate and oil was investigated to determine whether or not the combination of solute and solvents was significantly more toxic than the solute alone. It was not the purpose of this investigation to determine the toxic levels of the solvent.

One gram of 2,4-D is soluble in 2 ml. of tributylphosphate and the resulting solution is miscible in all proportions in oil. Various concentrations of the agent in tributylphosphate and oil were prepared by altering only the amount of oil used and leaving the agent-solvent ratio constant. The first two experiments on the 2,4-D tributylphosphate oil complex were preliminary in nature and consisted of a feeding titration in white rats using 1 per cent 2,4-D and 2 per cent tributylphosphate in oil, and a feeding titration in guinea pigs using 4 per cent 2,4-D and 8 per cent tributylphosphate in oil. It can be seen from Table 6, which summarizes the results of the first two experiments, that when a 1 per cent solution was used deaths occurred at one-fifth the tolerated dose of 2,4-D and all control animals died, whereas, when a 4 per cent solution was used, only occasional deaths occurred in the control group and in the series of animals fed the tolerated dose. In view of the results of the first two experiments it was decided to test a constant amount of 2,4-D (the tolerated dose) in various amounts of tributylphosphate and oil. For this work, concentrations of 1 per cent, 2 per cent and 4 per cent 2,4-D in oil containing 2 per cent, 4 per cent, and 8 per cent tributylphosphate respectively, were used for feeding experiments with guinea pigs, white rats and rabbits.

When a 4 per cent solution of tributylphospbate in oil containing a tolerated dose of 2,4-D was fed to experimental animals in a comparatively small volume of oil, all or most of the animals survived. Only occasional deaths were observed in the groups of guinea pigs and white rats, and all of the rabbits survived. When the 2 per cent solution was used,

TABLE 5

A Comparison of the Toxicity of Pure and Crude Ammonium Salt with the Pure Sodium Salt of 2,4-D

Species	mg./kg.	Route	Mortality***			
			Ammonium Salt		Sodium Salt Pure	Controls
			Pure	Crude		
White mouse	500	I.P.*	9/10	7/10	10/10	
	375	I.P.	4/10	3/10	3/10	
	250	I.P.	0/10	1/10	0/10	
	125	I.P.	0/10	0/10	0/10	
	Saline control	I.P.				0/10
White rat	333	Feed**	0/6	0/6	0/6	
	Saline control	Feed				0/6
Guinea pig	33	Feed		0/6	0/6	
	Saline control	Feed				0/6
Rabbit	200	Feed	0/4		0/4	
	Saline control	Feed				0/4
Monkey	285	Feed	0/1			

* I.P.—Intraperitoneal injection of 2,4-D salts in physiological saline solution.

** Feed—Feeding by stomach tube of 2,4-D salts in physiological saline solution.

*** Mortality data are recorded as ratio of deaths to total animals used.

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deaths were still sporadic but occurred with greater frequency than with the 4 per cent solution. However, when the 1 per cent solution was used, the tolerated dose in the large amount of oil killed all of the animals fed with the mixture. All of these experments pointed to the fact that the volume of oil and tributylphosphate fed governed the results of the toxicity tests, and that the 2,4-D was secondary in importance. There was no evidence of any additive effect between the 2,4-D and the tributylphosphate-oil portion of the complex since deaths occurred in the tributylphosphate-oil control groups to about the same extent as in the groups of animals fed the same volume of 2,4-D tributylphosphate mixture. No attempt was made to determine whether the toxicity of the tributylphosphate oil mixture was due to the tributylphosphate or the oil. In view of the comparatively slight differences in toxicity between the saline and oil solutions of 2,4-D in concentrations greater than 2 per cent, it was decided to test a 3 per cent solution of 2,4-D in oil and to feed various species ¼ of the tolerated dose. Such an experiment was carried out in white rats, guinea pigs, and rabbits, and all animals survived. The solution used was 3 per cent 2,4-D and 8.4 per cent tributylphosphate in #2 diesel oil. The amount of this material tolerated by the experimental animals was equivalent to about 200 cc. for the average sized man. One monkey was fed an amount of 4 per cent 2,4-D in oil containing 0.5 grams 2,4-D. This volume of material, 12.5 cc., caused very violent regurgitation soon after the feeding was carried out, but the animal was apparently normal the next day. No symptoms other than the nausea were noticed.

Three experiments were carried out to test the toxicity of n-butyl alcohol solutions of 2,4-D. These experiments were similar in nature to those carried out with the tributylphosphate-oil solutions. Two groups of rabbits were fed the tolerated dose of 2,4-D (0.5 gram) in 2 per cent and 4 per cent solution and all animals survived. In comparable experiments in guinea pigs and white rats, only an occasional death occurred. Occasional deaths were also observed in the control groups which received comparable volumes of n-butyl alcohol-oil mixture. Although not conclusive, these experiments indicated that n-butyl alcohol oil solutions of 2,4-D are at least of the same order of toxicity as the tributylphosphate oil solutions.

TABLE 6

TITRATION OF ONE PER CENT AND FOUR PER CENT 2,4-D IN TRIBUTYLPHOSPHATE AND OIL IN WHITE RATS AND GUINEA PIGS BY FEEDING

SPECIES	PER CENT 2,4-D	AMOUNT 2,4-D FED	VOLUME OF SOLVENT	MORTALITY**
White rat	Oil—T.B.P. (2%) control	None	5.0 ml.	4/4
	Oil control	None	5.0 ml.	4/4
	1	10 mg.	1.0 ml.	2/4
	1	25 mg.	2.5 ml.	3/4
	1	*50 mg.	5.0 ml.	4/4
	1	100 mg.	10.0 ml.	4/4
Guinea pig	Oil—T.B.P. (8%) control	None	2.5 ml.	1/4
	Oil control	None	2.5 ml.	0/4
	4	25 mg.	0.625 ml.	0/4
	4	50 mg.	1.25 ml.	0/4
	4	*100 mg.	2.5 ml.	1/4
	4	200 mg.	5.0 ml.	4/4
	4	300 mg.	7.5 ml.	4/4

* Tolerated amount of 2,4-D when in saline solution.

** Mortality data are recorded as ratio of deaths to total animals used.

Sub-acute Intoxication in Dogs

A group of 8 dogs received 2,4-D intravenously, varying from two injections of 200 mg./kg. each to six injections of 25 mg./kg., the injections being given at daily intervals.

Two dogs (nos. 1 and 2) receiving two injections each of 100 mg./kg. died on the third and second

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day respectively after the last injection. The first symptom was an unsteady gait, with the extremities somewhat stiff and extended. On the day following injection both animals were lethargic and stood up only when forced. The gait was still unsteady and extremities stiff. The deep reflexes of both animals were equal and active, pupils in mid dilation and equal. Corneal reflex in dog no. 1 was absent in contrast with dog no. 2. Nictitating membrane was sluggish in dog no. 1 covering one-half of the eye ball; it was active and in normal position in dog no. 2.

Two dogs (nos. 3 and 4) received two injections of 200 mg./kg. each on successive days. Both animals died on the second day. They were semi-stuporous for the first day after injection and responded slowly to stimulation. Pupils did not react to light and corneal reflex was either absent or extremely sluggish. Deep reflexes were equal and active in dog no. 3. The polymorpho-nuclear leucocyte count was 13,000 before injection and 1,400 the day following injection. Similarly, the lymphocyte count decreased from 3800 to 800. Dog no. 4 showed no significant change in the number of polys but did show a reduction of ymphocytes from 5,000 to 2,200.

One dog (no. 6) was given six injections at daily intervals of 25 mg./kg. of 2,4-D. On the second day this animal showed blood oozing from the gums at the tooth margins which did not increase in severity. On the fifth day after injections were terminated, decubitus ulcers developed over the outer aspect of the forelegs and right thigh which gradually became more extensive, deeper and infected. Neutropenia or lymphopenia were not apparent at any tme during the illness. The animal was sacrificed on the ninth day.

Two animals (nos. 7 and 8) received six injections, at daily intervals, of 50 mg./kg. each. Dog no. 8 died on the third day. This animal became lethargic and semi-stuporous after the third injection. On the second day after the series of injections were terminated conjunctivitis developed. Blood oozed from the gums and tooth margins, the gait became unsteady and coarse muscle tremors appeared in the extremities after moderate exercise. The deep reflexes were equal and active. The rectal sphincter was lax and allowed the escape of light, watery brown stools. The animal became more lethargic on the third day and died in the mid-afternoon. Polymorpho-nuclear leucocyte count decreased from 6,000 to 2,500 and the lymphocyte count from 3,500 to 200 as a result of the intoxication. The platelet count, originally 323,000, dropped to 138,000 in the post-injection period. Dog. no. 7 developed necrosis of the gums on the right and left side of the lower jaw along the posterior aspect where the teeth of the upper jaw overlap those of the lower jaw. Blood oozed from the gum margins throughout the remainder of the mouth. The necrotic process gradually extended to involve the floor of the mouth, the buccal mucous membrane and exposed much of the mandibular bone. Decubitus ulcers developed on the outer aspect of the extremities on the 14th day. The animal was sacrificed on the sixteenth day when rectal temperature was elevated for the first time. This animal did not show any significant reduction in the polymorpho-nuclear count. There was, however, a reduction in the lymphocyte count from 2,600 to 1,100.

It was interesting to note that smaller doses of 2,4-D appeared to have a cumulative action. The development of the decubitus ulcers and severe necrotizing lesions of the mouth occurred in all animals receiving 2,4-D over a protracted period. In general, the animals showed a slight reduction in the red count, hemoglobin level, and an increase in the plasma non-protein and urea nitrogen. There was no significant change in the total protein content of the plasma. At no time did any of the animals show any signs of icterus.

Sub-acute Oral Toxicity in Rats

Young male rats, weighing around 100 gms. each, were put in individual metabolism cages and fed a stock diet of sucrose, Labco casein, Crisco, and salts. In addition they received a daily vitamin supplement containing pyridoxine, riboflavin, nicotinamide, calcium pantothenate, choline chloride and thiamine. Following an observation period of one week, the animals were divided into 4 groups of 7 rats each. Group 1 received 100 mg. of 2,4-D/kg. of diet; Group 2, 200 mg.; Group 3, 400 mg.; Group 4 served as controls.

No difference could be observed in the food consumption and growth rate of animals receiving 100 and 200 mg. of 2,4-D/kg. of diet. One animal in the group receiving 500 mg. of 2,4-D of diet died on the tenth experimental day, but in as much as no other member of the group was affected, the death was considered incidental.

In view of the negative nature of the experiment, the diet of the rats of group 1 was changed on the

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twenty-first experimental day to contain 1000 mg. of 2,4-D/kg. of diet. Despite this large dose there was no evidence of reduced food intake and no effect on the growth rate during the subsequent two weeks. The experiment was discontinued one month from the time 2,4-D was first introduced into the diet. It was concluded that the rats' diet containing as much as 0.1 per cent 2,4-D was not intoxicating.

Sub-acute Oral Toxicity in Guinea Pigs

Two groups of 6 guinea pigs each were fed daily 50 mg. and 100 mg. respectively of the purified sodium salt of 2,4-D. On two occasions it was impossible to carry out the feedings on schedule so that the animals actually received only ten feedings in twelve days. Five out of 6 of the guinea pigs receiving 50 mg. at each feeding survived. These 5 animals were fed a total of 0.5 gm. in twelve days. Three out of 6 of the guinea pigs receiving 100 mg. at each feeding survived. The total dosage of this group was 1.0 gm. in twelve days. These results are nullified to a certain extent by the fact that three deaths occurred in the saline controls, making it appear that all deaths might have been non-specific and caused solely by the rather rough treatment associated with daily feeding by stomach tube. Supporting this contention is the fact that none of the animals showed the typical complete paralysis before death. Aside from these discrepancies, the fact remains that 3 guinea pigs survived a total of 1.0 gm. of 2,4-D, which is about 3 or 4 times the amount necessary to kill when given at one feeding.

The Toxicity of the Sodium Salt of 2,4-D by Inhalation

Attempts were made to produce symptoms in guinea pigs by exposing them to a wet cloud of sodium salt of 2,4-D in a spray chamber produced by nebulizing an aqueous solution of the salt and to a dry cloud of the crude acid produced by directing a jet of air against a finely pulverized dry powder in the bottom of a small chamber. In no case did any of the animals exhibit typical symptoms and there was no gross evidence of lung irritation, even though concentrations of the material in the air were high and exposure times were long. In the first experiment carried out in the spray chamber the animals were exposed to a CT (mg. min. per M^3) of approximately 6000–8000. The second experiment was not quantitative in that it was impossible to calculate the CT given. It has been estimated that the animals were exposed to a CT even greater than used in the spray chamber. No data was obtained on the average size of the particles in the aerosols or the fraction of the material impinged out of the inspiratory air and absorbed by the animal.

Pathological Changes

Rats and guinea pigs dying of massive doses of 2,4-D were sacrificed sixty to ninety-six hours after administration of the chemical were autopsied (the central nervous system was not examined). Constant findings on gross examination were congestion of the viscera and enlarged swollen kidneys. On section of the kidneys, the parenchyma bulged from the cut surface and the capsule stripped almost spontaneously presenting a dull yellowish-red surface. The cortex was swollen and poorly differentiated from the medulla. Microscopic examination of the kidney revealed the swollen cortex to be due almost entirely to a massive clouding swelling of the epithelium of the proximal convoluted tubules, which in many cases completely occluded the lumina. Some of the cell membrane had ruptured, with the escape of the cytoplasm and nuclei to form an acidophilic debris in the tubules which was later converted to hyaline and cellular casts found in the collecting tubules. The glomeruli and blood vessels were unaffected. Occasional animals showed slight patchy pulmonary edema and alveolar hemorrhages. The livers did not show any significant pathological changes.

A group of 6 rabbits receiving from four to thirteen daily (except Sunday) injections of 50 mg./kg. were sacrificed on the day of or the day following the last injection. No striking pathological changes were observed in this group of rabbits. The kidneys of all animals but one showed slight to moderate degenerative changes consisting of granular or vacuolar swelling of the cells of the proximal convoluted tubules. The glomeruli were unaffected. Stimulation of hematopoietic and reticulo-endothelial tissues was suggested by the occurrence of hyperplasia of the lymph nodes and spleen in several animals. Moderate hyperplasia of the bone marrow associated with the extra-medullary hematopoiesis in the liver, spleen, and adrenals. This effect was not consistent throughout the group. Significant blood destruction, evidenced by considerable

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hemosiderosis of the spleen, was present in only one animal. A review of the blood hemoglobin levels in this animal showed a decrease in the hemoglobin from a pre-injection level of 9.0–9.7 gm. to 7.8 gm. after five injections. Of three other rabbits in this group receiving as many or more injections, two showed no significant change in hemoglobin level and one showed a slight decrease. The liver showed no significant changes. The lungs showed scattered petechial hemorrhages in a few cases and moderate patchy edema in two.

Another group of 6 rabbits receiving from three to seven daily injections of 100 mg./kg. were sacrificed on the day of or the day following the last injection. There was a significant decrease in hemoglobin and red cells in one animal of this group, and a moderate decrease in hemoglobin in three others. No changes in the white cell count or differential count were observed. The kidneys of this group of animals also showed parenchymatous degeneration as the most constant change. Marked hyperplasia of the bone marrow, associated with extra medullary hematopoiesis, occurred in one animal. Lymphoid and splenic hyperplasia are observed in several animals but was not a constant finding. Hemosiderosis of the spleen was observed only once, in the animal showing the considerable decrease in hemoglobin and red cells. Evidence of slight lymphoid destruction was observed in the spleens of two animals of this group. The liver showed no significant changes.

Dogs succumbing to massive doses of 2,4-D or sacrificed were autopsied. In contrast with the other species studied, dogs showed a considerable susceptibility to the development of liver damage. The hepatic lesions consisted of centro-lobular degeneration, atrophy and lysis of the parenchymal cells about central veins of the lobules, with congestion and dilatation of the pericentral sinusoids. In one dog, dying at thirty hours after two injections of a relatively high dose (200 mg./kg.), there was an extensive though early necrobiosis throughout the liver.

The kidneys showed cloudy swelling of the tubular epithelium; the reversible nature of this lesion is indicated by its absence in animals sacrificed or dying after some delay after the last injection. On the other hand, one dog receiving a large dose (400 mg./kg.) showed actual tubular epithelial necrosis, similar in type to that observed in mercury poisoning. Striking lymphoid necrosis the lymph nodes, thymus and spleen was evident, especially at the higher doses. Unequivocal bone marrow changes were not observed, although it seems quite possible that with the proper dosages of the chemical such lesions might occur. An occasional animal showed evidence of blood pigment deposition in the spleen and lymph nodes. Minor focal parenchymal lesions were observed in the adrenal in several instances, accompanied by infiltration of the polymorphonuclear leucocytes.

Discussion

The investigations disclosed that 2,4-D is a relatively non-toxic compound for experimental animals, having an LD_{50}, expressed in mg./kg., of 375 for mice, 1000 for guinea pigs, 666 for rats and 800 for rabbits when administered orally in aqueous solution. All of these species reacted similarly to the chemical. There was no apparent difference in the toxicity between crude acid and a highly purified preparation, or between the sodium and ammonium salts of the acid.

Monkeys were shown to be able to tolerate 428 mg./kg. of 2,4-D when administered intraperitoneally. However, when the material was given in large doses by mouth, 1 or 1½ grams, the animal became nauseated and vomited a large portion of the material so that accurate information as to the toxicity of the compound administered by the oral route in this species is lacking. Three-fourths of a gram of the material was given to one monkey without the development of vomiting or serious illness. Based on the best data available, which are recognized to be inadequate, monkeys can tolerate single dosages equivalent to 214 mg./kg.

In any assessment of the acute toxicity of a chemical based on data obtained from laboratory animals it should be borne in mind that considerable variation in species susceptibility may occur and that the data obtained cannot always be translated into the toxic doses for humans. In this case, however, all of the laboratory animals tested reacted in a similar fashion to the material so far as could be determined from signs and symptoms which developed and from the pathological lesions which were present at autopsy. Assuming that man is no more resistant or susceptible than the rabbit or monkey, then the largest tolerated dose for a 75 kg. man would be 15 gms. With the exception of the monkey, all of the laboratory animals used lacked the vomiting reflex so that they were unable to relieve them-

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selves of irritating material by vomiting. The experiments conducted in monkeys indicate that the material is a gastric irritant in large doses, so that the possibility of the occurrence of acute poisoning in humans would seem relatively remote because of the large dose which man could presumably tolerate. Assuming that man is no more susceptible than the most susceptible animal tested, the mouse, then the calculated oral LD_{50} for man would amount to approximately 28 gms.

Sub-acute intoxication was produced in dogs by giving daily injections of the material over a period of six days. With the administration of smaller doses, 25 mg./kg., there was a suggestion that the material had a cumulative action. In contrast with the other species studied, dogs showed a considerable susceptibility to the development of liver damage. Occasional lymphoid necrosis in the lymph nodes, thymus, and spleen occurred especially with high doses. A few animals demonstrated a significant reduction in the number of lymphocytes in the circulating blood. The development of decubitus ulcers and their occasional association with a significant reduction in the leucocyte count was a disturbing observation. Reduction in the leucocyte count did not occur in every case, but its occasional development should direct attention to blood studies in cases of suspected intoxication in man.

Investigations on the sub-acute toxicity of 2,4-D by the oral route were limited to two species, rats and guinea pigs. Rats were fed varying amounts of the material up to $\frac{1}{10}$ per cent by weight of their diet for a period of one month without any significant effect on their food intake, rate of growth, or the development of any characteristic signs of intoxication. Guinea pigs, which were fed 100 mg. per day of the material by stomach tube over a period of twelve days until a total of 1 gram of the material had been administered, did not develop characteristic evidence of intoxication. In this particular experiment, non-specific deaths occurred in approximately the same percentage in both the test and control animals. All of these deaths were believed to result from trauma, associated with the passage of a stomach tube at frequent intervals, in as much as none of the test animals developed the typical paralyses or skeletal muscular signs observed in poisoned animals. It was therefore concluded that guinea pigs could tolerate 1 gram of 2,4-D in divided doses over a period of twelve days without any harmful effects.

The experiments to determine the toxicity of the sodium salt of 2,4-D by inhalation tended to indicate that the material was relatively non-toxic when wet or dry clouds were inhaled. It should be pointed out that in none of these experiments was the average particle size of the aerosol determined nor the amount of material retained and absorbed by the respiratory tract determined. Further studies on the toxicity of this compound by the respiratory portal of entry will be needed before any final conclusions can be made. In view of the fact that the material did not produce any evidence of lung irritation and that the toxic oral dose of the material for guinea pigs is relatively high, it would tend to indicate that the material would be relatively non-toxic by this route.

2,4-D dissolved in a solvent complex of tributylphosphate and diesel oil has been recommended for use as a herbicide. This particular form of material has the advantage over the aqueous solution in that the tributylphosphate acts as a co-agent or synergist to the 2,4-D producing greater plant damage than could be accounted for by the acid alone. The present study did not attempt to determine the toxic or tolerated dose of tributylphosphate or diesel oil.

There was no evidence of a synergistic or additive effect when a tolerated amount of 2,4-D was dissolved in the tolerated dose of tributylphosphate oil complex and administered to the experimental animals. Production of chronic poisoning by this material was not attempted.

Summary

The LD_{50} expressed in mg./kg. of 2,4-dichlorophenoxyacetic acid by mouth is 375 for mice, 666 for rats, 800 for rabbits, and 1000 for guinea pigs. The tolerated dose for these same species was 125, 166, 200 and 333 mg./kg. respectively. The largest dose administered to monkeys without serious after effects was 214 mg./kg.

Subacute intoxication was produced in dogs by the daily administration of 25 mg./kg. over a six day period with the development of liver damage. Leucopenia was observed in a few animals.

Rats were fed up to $\frac{1}{10}$ per cent by weight of their diet over a period of one month without any harmful effects. Guinea pigs can apparently tolerate 1 gram of the material administered in divided doses of 100 mg. over a period of twelve days.

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A limited amount of work on the toxicity of wet and dry clouds of the sodium salt indicated that it was relatively non-toxic.

The toxicity of 2,4-D dissolved in tributylphosphate and diesel oil was not enhanced by these solvents.

ACKNOWLEDGMENT

The authors wish to thank Major Arthur Ginzler and Captain Morton Galdston of the Medical Division, Edgewood Arsenal for permission to use their data on subacute toxicity of 2,4-D for dogs and chronic toxicity in rats.

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BIOCHEMICAL RESEARCH LABORATORY
THE DOW CHEMICAL COMPANY

Subject TOXICOLOGY AND HYGIENE:
2,4-DICHLOROPHENOXY ACETIC ACID

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Att: B. N. Schrauf
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Safety Dept.
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Dr. H. H. Gay
T2.2-10-1

Check 6-30-48

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UNIT INDEX

A summary of available information.

INDEX HEADINGS

Acetic acid, 2,4-dichlorophenoxy -

SUMMARY OF HAZARD

The toxicity is such as to present but minor hazards of systemic effects. Skin irritation is possible from heavy prolonged contact with strong solutions or the solid acid. There is no hazard of absorption through the skin.

SUMMARY OF TOXICITY

The systemic toxicity was moderately high as indicated by single and repeated oral feedings to rats.

For single doses:

Largest dose survived by all rats = 0.3 g./kg.
Smallest dose to which all rats succumbed = 1.0 g./kg.

For repeated (20) doses; 0.3 g./kg. rapidly produced serious injury and death, 0.1 g./kg. had but very slight deleterious effect and 0.03 g./kg. had none.

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Examination of the affected animals revealed minor effects in liver and kidneys, the major action being local irritation in the stomach and gut.

When incorporated into the diet of rats, there was no adverse effect at concentrations which the rats would eat, 0.03% and less. At concentrations of 0.1% and more, the rats refused to eat.

The dichlorophenoxyacetic acid had but very slight effect when tested in solution upon the skin of animals.

There was no evidence of absorption through the skin.

In making and processing 2,4-dichlorophenoxyacetic acid there appears to be no serious hazard of systemic effects. Atmospheric contamination by dust offers the only concern and this would have to be quite severe to be excessive. Any such excessive exposure would probably be first indicated by symptoms referable to irritation in the upper respiratory passages or by gastrointestinal disturbances. By comparison with other common substances, it offers very little hazard of serious organic injury, even from repeated exposures.

Animal results indicate little irritating action upon the skin. There is the possibility that prolonged heavy exposures to solid or to strong solutions could cause a dermatitis. This applies to both the free acid and the sodium salt.

In the use of 2,4-dichlorophenoxyacetic acid, the solution concentrations are sufficiently low and the total amounts involved sufficiently small that there appears to be no hazard to the applicator or to farm animals.

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of isometric recordi.., the movement of the pin under a full load of 1500 g. is limited o 0.04 mm. The bridge signal was fed through a direct-coupled amplifier to a athode ray oscilloscope for photographic recording. The bridge output was linear hrough full-scale deflection. Resting tension of the muscle was maintained at a onstant value throughout any one experiment, but varied from one experiment to nother between 75 and 200 g.

For indirect stimulation the sciatic nerve was crushed and tied proximally. The istal segment was placed in a plastic insulating bed containing two silver stimulating ectrodes. Direct stimulation was effected through steel needles placed at opposite ıds of the muscle. Stimuli, which were supramaximal in intensity, were presented s square waves generated by a trigger circuit, the duration of which was controlled y a variable resistance-capacitance circuit.

Nerve action potentials were recorded through a Sherrington electrode from the bial nerve isolated peripherally. Stimulation was effected at the pelvic end of the iatic nerve which was isolated centrally.

Electromyograms were recorded through steel needles insulated to within 1 to 2 m. of their tips and placed in the belly of the muscle approximately 5 mm. apart. he potentials were fed through a condenser-coupled amplifier to the cathode ray scilloscope and recorded photographically. When the needles were inserted shortly ter section of the sciatic nerve, spontaneous random spike potentials were observed. hese spikes lessened in frequency and then disappeared in the course of 10 to 15 inutes. No studies were carried out until spontaneous activity had subsided.

Intra-arterial injection was made through a small cannula tied into the contra-teral iliac artery and directed centrally. The crural artery of the leg under study as ligated. The aorta was occluded during injection by drawing up on a loose gature placed above the bifurcation. The injection volume was 0.15 ml. This eparation preserved normal blood supply to the muscle under study but is under ntinuing development to attain further restriction of the injection to the triceps rae. The small size of the rat's peripheral vessels enabled the use of a simple high-equency desiccating apparatus ('Hyfrecator', Bircher) to coagulate vessels and, ius, to reduce bleeding.

When required, denervation of the triceps surae was performed 10 days before cording by aseptic section of the sciatic nerve.

Concentrations of potassium in serum and muscle were determined in control its of the same weight in whom equivalent amounts of KCl had been injected intra-eritoneally (i-p). Analyses were made in a modified Berry-Chappell-Barnes in-rnal standard flame photometer (9, 10).

Prior to administration of curare the trachea was cannulated and artificial res-iration provided by a pump.

The concentrations of the agents used and their dosages follow:

1) Sodium pentobarbital, 25 mg/ml., was administered i-p in doses of 40 to 50 mg/kg. body eight.

2) Sodium 2,4-dichlorphenoxyacetate monohydrate (Baker) was dissolved in water in a con-ntration of 50 mg/ml., and the *p*H was adjusted to 7.4 with dilute HCl for i-p injection in doses of 0 to 250 mg/kg.

3) *d*-Tubocurarine chloride[3] was injected i-p in doses of 1.5 mg/kg. This is equ[illegible]t approximately to one unit of curare per animal and produced total paralysis of all skeletal muscle.

4) KCl was injected i-p as a 20 mg/ml. (270 mEq/l.) aqueous solution. Doses were 400 mg/kg. (5.4 mEq.).

5) Quinine dihydrochloride was injected intramuscularly in aqueous solution of 45 mg/ml. and in doses of 150 mg/kg.

6) Disodium d-l-α-tocopheryl phosphate[4] was injected i-p in an aqueous solution of 100 mg/ml. in doses of 1 g/kg.

7) Magnesium sulfate was injected i-p in doses of 250 mg/kg.

8) Calcium gluconate was injected i-p in doses of 100 mg/kg.

RESULTS

Our observations are in full agreement with those of Bucher on the behavior of the rat which has received 2,4-D (4). In both conscious and anesthetised rats full development of myotonia appeared some 30 to 45 minutes after i-p injection and lasted for hours. The injection of small amounts (2 mg.) of 2,4-D into the vascular tree was followed by extreme generalised myotonia in two minutes. Even under deep anesthesia and full curarisation, the resistance to passive motion of the extremities was appreciably enhanced.

Primary effects of 2,4-D on muscle function are illustrated in figures 1 and 2. Increase in tension developed by an isometric twitch, in response to a single supramaximal stimulus to the nerve, was of the order of 25 to 30 per cent; duration of twitch until half relaxation was greatly prolonged (figs. 1A and 2A).

The electromyogram of normal muscle stimulated by a single shock to the nerve is a simple diphasic deflection (fig. 1B). When myotonia had developed after injection of 2,4-D the response became repetitive. The rapidly recurring, brief potential changes were less than the initial spike, and occasionally they did not appear until after a short period of electrical silence, 10 to 50 msec., following the initial spike. On one occasion the silence persisted for 600 msec. (fig. 3C). In some records the regular rhythmicity of the repetitive response suggested that a single motor unit was firing directly under the recording electrodes. The duration of repetition was variable, ranging from 100 msec. to 6 sec.

Prolongation of twitch and repetitive firing decreased rapidly with repeated stimulation; at a stimulus rate of 12 per min. myotonic features diminished rapidly during the first five or six consecutive single volleys (fig. 1A, B). Rest for 10 minutes resulted in complete return of the myotonic response. The same changes were noted following stimulation by a pair of nerve volleys delivered at short intervals.

Myotonia in man and goat is characterised by exquisite sensitivity of muscle to mechanical stimuli. 2,4-D produced in the rat this same explosive electrical response to tapping of the muscle or tendon and also to the insertion or movement of the recording electrodes (fig. 2).

The site of development of the myotonic response to 2,4-D was delimited partially by injection of sufficient *d*-tubocurarine chloride to block completely any muscle

[3] Generously provided by E. R. Squibb and Sons, New York, N. Y., and by Abbott Laboratories, North Chicago, Ill.

[4] Generously provided by Hoffman-LaRoche, Nutley, N. J.

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these conditions, direct electrical stimulation of the muscle produced increased twitch tension, prolongation of the contraction and repetitive response indistinguishable from that observed in curarized muscle (fig. 3). The triceps surae, denervated 10 days before examination, also developed myotonia in response to 2,4-D despite obvious atrophy.

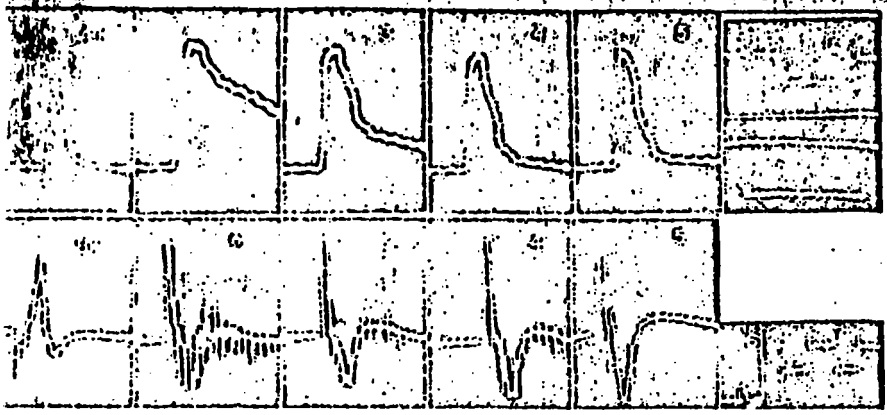


Fig. 1. INDIRECT STIMULATION OF THE TRICEPS SURAE BY THE SCIATIC NERVE. *A*. 1. Normal myogram. 2–5. Consecutive myograms, 120 min. after 40 mg. of 2,4-D, in response to stimuli delivered at a rate of 12/min., illustrating decreased duration and the phenomenon of 'warm-up'. 6. Time: 50 msec. 7. Tension: 200 g. *B*. 1. Normal electromyogram. 2–5. Consecutive records of *A*. 6. Voltage: 200 μV. 7. Time: 50 msec.

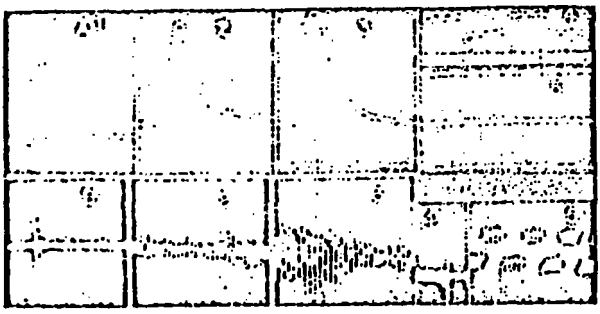


Fig. 2. INDIRECT STIMULATION AND MECHANICAL TAPPING. *A*. 1. Normal myogram. 2. 30 min. after 30 mg. 2,4-D. 3. 39 min. after 60 mg. KCl (same preparation as 2). 4. Time: 50 msec. 5. Lower line: zero tension; middle line: resting tension of 143 g.; upper line: 200 g. *B*. Electromyogram in response to a brisk tap on tendon. 1. Normal. 2 & 3. Corresponds to A2 and 3. Voltage: 1 μV. 5. Time: 50 msec.

The familiar simple diphasic electrogram of nerve stimulated by a brief single shock is recorded in figure 4 (11). One hour after administration of 2,4-D a similar single stimulus evoked a volley of potentials which arose in the nerve proper, since it had been isolated from both central and peripheral structures.

Certain agents which modify spontaneous myotonia were studied for their effects on the myotonia produced by 2,4-D. *Potassium*, 5.4 mEq/kg. i-p, elevated the serum concentration from a normal level of 5 mEq/l. to 10 to 14 mEq/l. in 20 to 30

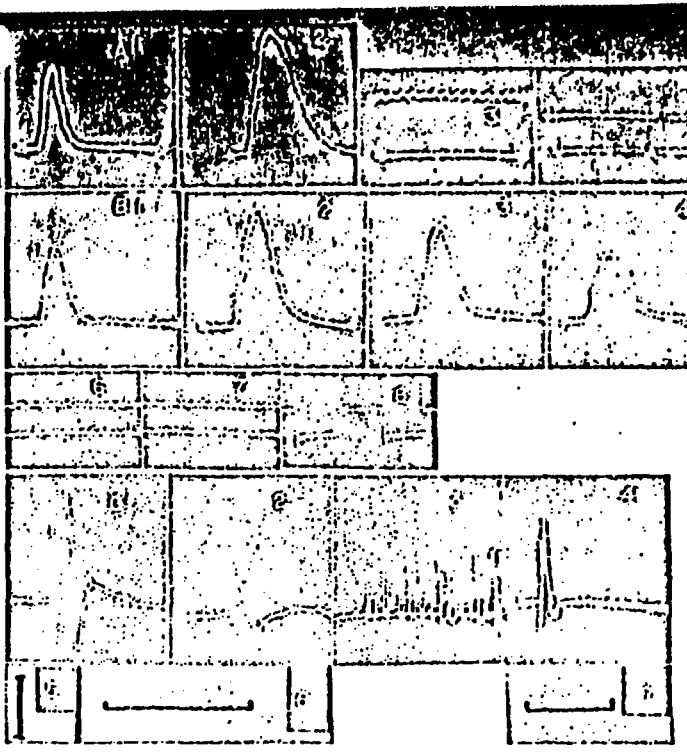


Fig. 3. COMPLETELY CURARIZED PREPARATION; DIRECT STIMULATION. *A*. 1. Normal myogram 2. 45 min. after 40 mg. 2,4-D. 3. Tension: 200 g. 4. Resting tension: 125 g. 5. Time: 50 msec. *B*. 1. Normal myogram. 2. 45 min. after 40 mg. 2,4-D. 3. 11 min. after 20 mg. quinine. 4. 5 min. after additional 20 mg. quinine. 5. 10 min. after 4. 6. Tension: 200 g. 7. Resting tension: 200 g. 8. Time: 50 msec. *C*. 1. Normal electromyogram. 2 & 3. Consecutive sweeps 40 min. after 30 mg. 2,4-D, illustrating an unusually prolonged period of silence (600 msec.). 4. 5 min. after 40 mg. quinine (irregularity of trace is owing to 120 cycle interference). 5. Voltage: 1 μV. 6. Time: 50 msec. (referring to 1–3). 7. Time: 50 msec. (referring to 4).

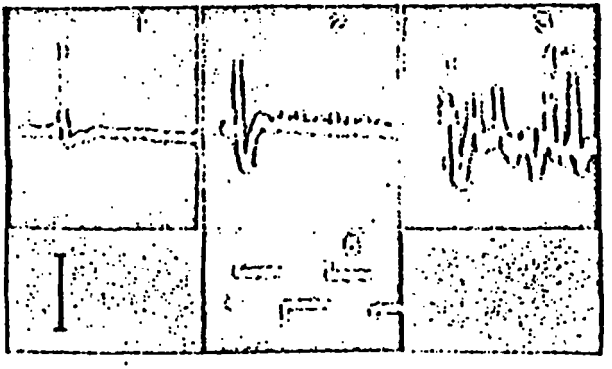


Fig. 4. DIPHASIC ACTION POTENTIAL FROM TIBIAL NERVE. 1. Normal. 2. 15 min. after 30 mg. 2,4-D. 3. 60 min. after 2,4-D. 4. Voltage: 200 μV. 5. Time: 50 msec.

minutes. This increase in serum concentration presumably was reflected rapidly in the interstitial fluid surrounding the muscle cells, but analyses of the total muscle itself revealed no significant increase in total K content. We have confirmed

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...ding to the normal rat this distortion of K^+ relationships resulted ...ghtened and lengthened tension response to both direct and indirect stimu... ...n and in repetitive firing of the muscle in response to a single stimulus (12). The ...infusion of KCl to a rat in full course of response to 2,4-D produced a further ...ntiation of the myotonic response and in degree of repetitive firing (fig. 3B).

Increased sensitivity to *acetylcholine* was demonstrated by injecting 100 μg. intra-...rially (11). In the preparation described here, this injection elicited a few small ...tered spike potentials in the electromyogram. After treatment with 2,4-D the ...e amount of acetylcholine evoked an explosive and prolonged burst of electrical ...vity.

Quinine has been shown to reduce or obliterate the myotonic response occurring ...taneously in man (13, 14) and goat (6, 8). The same effect was produced on ...otonia induced in the rat by 2,4-D. The myogram revealed a lowering of tension ...owing administration of quinine (fig. 3B). There are certain characteristics of ... response which warrant noting. First, tension developed after the second dose ...uinine fell to 54 per cent of the control twitch; and second, the duration of the ...ch was not reduced. The effect of quinine on the responses induced by 2,4-D ...own in figure 3C where the characteristic repetitive firing of the muscle was ...terated.

Alpha-tocopheryl phosphate, when injected i-p in relatively large doses into normal ..., produces profound generalized effects: somnolence, ataxia, muscular flaccidity, ...a and, occasionally, death following convulsions (15). When administered per-...ly to two patients with myotonic dystrophy a suggestive decrease in spontaneous ...otonia was observed (16). Injection of α-tocopheryl phosphate in the rat, which ... received 2,-4D, obliterated the evidences of myotonia as effectively as ...quinine.

Similar suppression of repetitive response followed injection of *magnesium* and ...*um*.

DISCUSSION

The neuromuscular apparatus of the rat, treated with 2,4-D, mimics faithfully ...characteristics of myotonia occurring spontaneously in man and goat. In both ...instances, muscle exhibits exquisite sensitivity and repetitive response to several ...ulating agents: motor nerve volley and direct stimulation, tapping, insertion of ...le electrodes, acetylcholine and K^+ (6). Furthermore, in both instances, re-...ted stimulation ('warm-up'), quinine, Mg^{++} and Ca^{++} diminish and obliterate ...repetitive phenomenon. Alpha-tocopheryl phosphate now may be added to the ...of agents inhibiting repetitive responses in both spontaneous and induced ...otonia.

These effects of 2,4-D, producing a myotonic reaction so reminiscent of the ...taneously occurring forms, are indistinguishable, likewise, from the consequences ...eatment with a variety of substances; *e.g.*, the 'veratrine' alkaloids derived from ...*trum* and *Schoenocaulon*, aldehydes, tetraethyl ammonium ions, phenanthrene-9-...oxylic acid and substituents, and dihydronaphthacridine carbonic acid

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(Tetrophan) (17). To this heterogeneous group we have added recently pentamethylene tetrazol (Metrazol) (18).

Many, and perhaps all, of these diverse veratrinic substances, however, produce the same repetitive response in nerve that they produce in muscle. It has not been established in spontaneous myotonia whether or not this repetitive phenomenon occurs in nerve as well as in muscle; nevertheless, some observations have suggested that myotonia in man is accompanied by functional changes not limited to muscle fiber proper (19). Indeed, it is reasonable to view repetition as a stereotyped response of excitable tissue (nerve, neuromuscular junction and muscle) to many unrelated alteratives which may play rôles of differing intensity on all excitable structures. A tentative exploration of this possibility might begin by examining the circumstances in which repetition has been observed.

In order to limit the analysis, only those instances of repetitive response to single stimuli have been considered; obviously, this limitation excluded other important changes in excitability manifested by spontaneous firing or by measurable changes in pre-discharge conditions, such as threshold, resting potential, recovery cycle etc. (20, 21). The appearance in excitable tissue of repetitive, rhythmic responses to a single stimulus occurs under the most diverse of circumstances, as for example: *a*) increased external hydrostatic pressure (22); *b*) stimulation by constant rectangular currents (23); *c*) increased external concentration of H^+ (20, 24), K^+ (12), acetate, lactate, citrate and oxalate (24), Ba^{++} and guanidine (25), adenosine triphosphate (26), DDT and quinoline (27); *d*) decreased external concentration of Ca^{++} (28); *e*) inhibition of cholinesterase activity at the neuromuscular junction by eserine (11), neostigmine (29) and DFP (30); *f*) tetanus toxin affecting the neuromuscular junction (31); *g*) treatment with that heterogeneous group of substances which produce veratrinic effects (17); and *h*) spontaneous myotonia in man and goat.

In the face of our fragmentary knowledge of processes subserving excitability, the incongruity of those conditions and agents producing repetitiveness makes it extremely difficult to apply any unifying concept which can explain all of the observations noted above. An example of the difficulties encountered in a simple approach is contained in an attempt to analyse the phenomenon of repetitiveness in terms of distortions of cationic *milieu*. Preservation of totally normal function depends on maintenance of concentrations of Na^+, K^+ and Ca^{++} within certain limits. Could these many agents act by disturbing the required cationic patterns? Simple inspection of the structure of the veratrinic agents reveals their variation through almost full scale with respect to chemical activity, steric and physical characteristics; some are potential metal sequestrants or precipitants, others totally inactive. Lack of any common characteristic is emphasized not to imply that a unitary pattern of action is non-existent but to stress the necessity for fitting many divergent observations into any proposed scheme.

It seems likely that these many different forces and agents may act at different loci in the complicated train of events responsible for the smoothly integrated flow of energy which maintains normal excitability. Only vague clues exist to suggest certain possible sites of action.

For example Lorente de Nó, from an elaborate analysis in frog nerve, suggests

ST?TTS[illegible] M00

...by ... the speed of or blocking some late step in the chain of ... processes interferes with the maintenance of the membrane potential.

From a study of plants and micro-organisms comes suggestive evidence that ...-D distorts mechanisms involved in the transfer of oxygen (32, 33). Compatible ...h, but not proving, this suggestion is the suppressive action of Mg^{++} on veratrinic ...enomena (24) and the similar action of α-tocopheryl phosphate which is so potently ...tioxidative as well as antiproteolytic (15).

Models of excitability or energy transfer expressed in terms of chemical or phys... structure, enzymatic activity, surface activity or membrane potential, may ex...in some or many of the observations; but none, as yet, has been offered in satis...tory interpretation of all the data.

The phenomenon of the silent period intervening between the normal single ...e potential and the outburst of repetitive spikes following treatment with 2,4-D the same as that described by Eichler in the frog treated with small amounts of ...ratrine' (34). A speculative interpretation of this phenomenon might assume ponential decay of facilitating and depressing processes initiated simultaneously the stimulus (35). If under the observed circumstances the depressing process ...ayed so rapidly that the facilitating process remained unopposed, then a burst of ...ivity might be released. The data available do not permit a more specific analysis.

SUMMARY

... 2,4-dichlorphenoxyacetate produces in the neuromuscular apparatus of the rat ...eratrinic response marked by repetitive response to single stimuli in muscle and ...ve. This results in increased 'twitch' tension and prolonged 'twitch' duration. ...e repetitive responses and their sequelae are accentuated by K^+ and acetylcholine ...d are obliterated by activity, quinine, Mg^{++}, Ca^{++} and α-tocopheryl phosphate. ...ese phenomena are indistinguishable from those occurring in the spontaneous myo...ia of man and goat, and also in response to several apparently unrelated chemical, ...ysical and electrical agents.

... We are deeply indebted to Dr. S. A. Talbot for designing and supervising construction of the ...ctronic instruments used in these studies.

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b

b

mild shock, respectively. All of the 10 controls showed shock, 2 died, 1 was severely shocked, and the rest showed moderate or mild shock.

Three weeks later some of the survivors of both the experimental and the control group were again challenged without the drug with 0.6 cc of egg white, I.V. All showed shock.

TABLE 1

EFFECTS OF ADMINISTRATION OF 15 GRAINS (.968 GM) OF ACETYLSALICYLIC ACID, IN DIVIDED DOSES, ON ANAPHYLACTIC SHOCK

No.	Dosage	Shock	21 days later
931	15 grains in 3 doses	0	..
935	"	0	3
937	"	0	4
939	"	0	1
941	"	0	..
943	"	0	..
945	"	0	..
947	"	0	..
949	"	0	..
953	"	2	..
955	"	1	..
933	None	3	4
934	"	2	4
936	"	1	1
938	"	1	..
940	"	4	..
942	"	2	..
944	"	2	..
946	"	1	..
948	"	2	..
954	"	4	..

To test the effect of acetylsalicylic acid on histamine shock, 11 rabbits weighing 2–2.5 kg were given 5 grains of acetylsalicylic acid, orally, at 3:30 P.M. on March 11, 1947. This dosage was repeated at 9:00 A.M. on March 12 and again at 2:30 P.M. the same day. One hour later, each rabbit was injected intravenously with 2.75 mg of histamine phosphate. Seven of the 11 died (#4 shock), 2 showed severe (#3) shock, and 2 showed moderate (#2) shock.

The animals which were premedicated with acetylsalicylic acid showed striking protection against anaphylactic shock. It may be presumed, on the basis of the literature reviewed above, that this was due to a decrease in the antibodies at the time of the challenging dose. There is nothing to indicate any protective effect against histamine shock, for though unaccompanied by simultaneous controls, the morbidity in the above series was similar to that reported in a previous communication (*1*). It is interesting to note that the effects of the drug were temporary and that the treated animals showed considerable hypersensitivity 3 weeks later.

The contrast between the results with this drug and those reported earlier (*7*) for Benadryl is complete. Benadryl gave good protection, in similar experiments, against histamine shock but was without effect on anaphylactic shock. The present drug, acetylsalicylic acid, however, effectively protects against anaphylactic shock but not against histamine. It is, with the other salicylates, apparently a true antianaphylactic drug in that it interferes with the antigen-antibody reactions to prevent or decrease the untoward results of the challenging dose of antigen.

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Effects of 2,4-Dichlorophenoxyacetic Acid on Chicks[1]

MELVIN K. BJORN and HENRY T. NORTHEN

Department of Botany, University of Wyoming

With the increasing use of 2,4-D as a herbicide it is important to inquire into the possible toxic effects of the chemical on animals. Several investigators have studied the effects of 2,4-D on mammals. The lethal dose for mice, when injected subcutaneously or intravenously, has been determined by Bucher (*1*) to be 280 mg/kg of body weight. Mitchell and Marth (*5*) reported that they fed 200 mg of 2,4-D daily to small experimental animals with no ill effects.

TABLE 1

EFFECT OF ALKANOLAMINE OF 2,4-D ON WHITE ROCK CHICKS

Dosage (mg of acid/kg of body weight)	Increase in weight at end of four weeks (%)
0.00 (control)	456
.28	444
2.80	469
28.00	427
280.00	373

In the present experiment White Rock chicks were used. The alkanolamine of 2,4-D was administered orally through a pipette. In the first experiment, data for which are given in Table 1, one part of the alkanolamine was diluted with 19 parts of water. The dosages recorded are in terms of the acid equivalent. Five chicks (each weighing approximately 50 gm at the beginning) were used in each group. The chicks were weighed and then were given the appropriate dose (Table 1) three times a week on alternate days for a period of four weeks, making a total of 12 doses. As the chicks gained in weight, the

[1] Contribution No. 210 from the Department of Botany and the Rocky Mountain Herbarium, University of Wyoming.

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amount administered was adjusted in order to maintain the original dosage. At the end of four weeks they were again weighed. The table shows the percentage increase in weight for the four-week period.

The differences between the control group and those given dosages of 0.28, 2.80, and 28.00 mg/kg were not significant at the 5% level. The difference between the control group and the group given a dosage of 280 mg/kg was barely significant at the 5% level.

Next, experiments were started to determine the lethal dose of the alkanolamine of 2,4-D when diluted 1:9 with water. Each chick of a group of 5 (each averaging 166 gm) was given one dose of 380 mg/kg of body weight. These chicks survived.

Each chick of another group of 5 chicks (average weight, 111 gm) was given a dose of 765 mg/kg of body weight. All of these chicks died. Postmortem examination revealed a fatty degeneration with a pale mottling of the liver, spleen, kidneys, and heart. Hemorrhagic gastroenteritis was also evident.[2] Hence, for small chicks the lethal dose of diluted 2,4-D is somewhere between 380 and 765 mg/kg.

The fact that a single dose of 765 mg killed, but not a total dose of 3,360 mg administered over a four-week period (280 mg/kg chicks of Table 1), indicates that the alkanolamine of 2,4-D is not a cumulative poison.

The question might be raised as to the possibility of chicks being killed by feeding on plants which had been sprayed with 2,4-D. At a spraying rate of 1 lb of 2,4-D/acre, a chicken weighing 1 kg would have to consume *all* of the 2,4-D applied on 72 sq ft within a day or two to obtain a lethal dose.

References

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A New Histological Procedure for Whole Tissue Cultures Grown in Plasma

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Chester Beatty Research Institute, Royal Cancer Hospital, Fulham Road, London, S.W.3

Standard textbook procedures (*2*, *3*) recommended for fixing and staining whole tissue cultures in plasma coagula have proven unsatisfactory for the following reasons: (1) The dense fixed coagulum presents a nearly impermeable barrier to the stain, thus necessitating prolonged staining periods; (2) the coagulum itself stains diffusely and heavily, preventing adequate contrast between the cells and the medium and necessitating careful and time-consuming decolorizing and developing procedures; (3) the resulting preparation, when mounted, is thick, has a tendency to form air bubbles, does not dry readily, and is generally inadequate for microscopic study. Tompkins, Cunningham, and Kirk (*4*) recently attempted to improve results by washing cultures in a salt solution for 4 hrs at 7° C to remove soluble protein before fixation, but still had to resort to prolonged staining (1 hr in Delafield's hematoxylin) followed by several hours of washing. Earle (*1*) has devised complicated fixing, staining, and mounting procedures, too elaborate to warrant evaluation here.

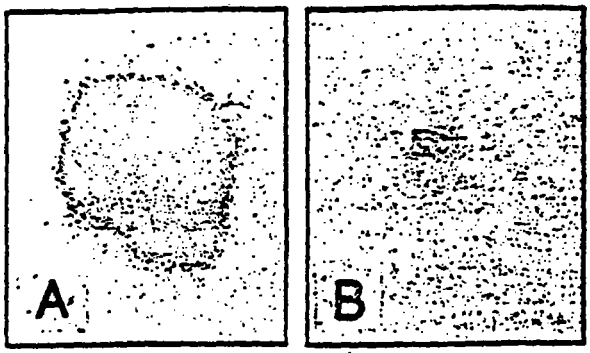


Fig. 1. Gross appearance of stained tissue cultures: A, plasma undried; B, plasma dried (× 1.5).

We have found that most of the disadvantages of the plasma coagulum can be eliminated by drying the whole tissue culture preparation after fixation. The procedure is as follows: The culture in its coagulum on a cover slip is fixed overnight in 3–4% formol containing 0.5% acetic acid or in Carnoy's fluid for 1 hr. In general, acetic-alcohol fixations give a more granular and more opaque dried specimen than acetic-formol fixations. The cover slip is then washed *thoroughly* in running water (after acetic-formol) or in descending strengths of alcohol, and water (after Carnoy's). After a final rinse in distilled water the cover slips are laid flat on a glass surface protected from dust (Petri dishes or staining dishes can be used) and allowed to dry slowly and thoroughly in air. If a preparation of a tube or a flask culture in plasma is desired, the portion should be fixed and washed *in situ*, pried loose and placed, like a paraffin section, in a dish of water, where it can be manipulated with a dissecting needle onto a slide or cover slip previously coated with a thin film of Mayer's albumin, and dried as above. Cultures grown in a fluid medium should not be dried. The dried cultures may be stained with any one of the hematoxylins for from 5 to 15 min, depending on the strength of the stain. Weigert's iron-hematoxylin has been found very satisfactory. The staining is simple to control after a preliminary trial, and prolonged washing to develop the color is not necessary. Once stained, the cultures should not be allowed to dry again. Following this, the cover slips are passed through the alcohols in the usual manner, cleared in xylene, and mounted. If a Leishman or Giemsa stain is used, acetone and acetone-xylene dehydrations should be employed instead of the alcohols. The dehydration and clearing procedures take considerably less time with dried cultures, since each

[4] The authors are grateful to Robert W. Lindenstruth, who made the examinations.

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Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

File T23.14-11-3
Chg. 1219-02
Rec'd 5/23/49
Fin'd 10/12/50
Work By V. K. Rowe

Subject: RESULTS OF SKIN IRRITATION TESTS ON VARIOUS SAMPLES OF 2,4-D TAKEN AT VARIOUS STAGES IN THE MANUFACTURING PROCESS

To
Britton's Division
Att: J. W. Britton
R. C. Dosser
C. A. Highhill
H. R. Hoyle ✓
Executive Research Committee
Organic Research Laboratory
Att: E. C. Britton

Check D W Hedges 10-13-50

Rept. By V K Rowe 10-12-50

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DOW 1 5493305
MN055656

UNIT INDEX

Impure 2,4-D is considerably more irritating than the purified product.

INDEX HEADINGS

2,4-Dichlorophenoxyacetic acid

Acetic acid: 2,4-dichlorophenoxy-,

PROBLEM

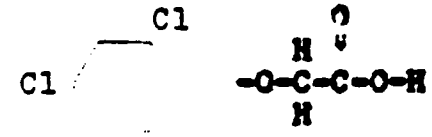
A few cases of dermatitis have been observed around particular operations in the manufacture of 2,4-D. Samples from the various stages of manufacture were submitted for skin irritation tests to determine whether or not there was a significant difference between the various preparations.

MATERIAL

Name: 2,4-Dichlorophenoxyacetic acid

Formula:

Structural:



Empirical: $C_8H_6O_3Cl_2$

Samples obtained by: H. R. Hoyle

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Sample description:

B.R.D. K no. 2372-12 is drier feed from plant no. 2, 267-Building containing 0.24% of 2,4-dichlorophenol.

K2372-13 is drier feed from plant no.1, 267-Building containing 1.21% of 2,4-dichlorophenol.

K2372-14 is unwashed cake from the filter box, 267-Building containing 0.96% of 2,4-dichlorophenol.

K2372-15 is cake from the Bird filter, 489-Building containing 0.10% of 2,4-dichlorophenol.

K2372-16 is an especially purified sample of 2,4-D obtained from 267-Building, low in 2,4-dichlorophenol.

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EXPERIMENTAL RESULTS

When the purified sample of 2,4-D, K2732-16, was repeatedly bandaged onto the shaven abdomen of a rabbit in a dry state, it produced no detectable irritation; when wet with water it produced only a very slight, simple irritation.

When the 2,4-D cake from the Bird filter, K2372-15, was repeatedly bandaged to the shaven abdomen of a rabbit, it failed to produce any appreciable evidence of irritation except a rather bluish coloration of the dermal tissue; when wet it produced a slight amount of superficial denaturation, again with a rather marked purplish coloration. This lesion healed promptly after exposures were terminated.

When the dry 2,4-D unwashed cake from the filter box in the 267-Building, K2372-14, was repeatedly bandaged to the shaven abdomen of a rabbit, it promptly produced an appreciable amount of irritation, characterized by hyperemia and exfoliation; when dampened with water this sample of material produced a moderate to severe

response. This latter response was characterized by moderate exfoliation and necrosis and a great deal of soreness.

When the 2,4-D drier feed from plant no. 1, 267-Building, K2372-13, was tested in a similar manner, it produced a slight, simple irritation, which was not enhanced by wetting the material.

When the drier feed from plant no. 2, 267-Building, K2372-12, was tested it produced no appreciable irritation.

CONCLUSIONS

It would seem from these results that impure 2,4-D is more likely to produce a significant amount of skin irritation than is pure material; and also, it seems that a damp product is more likely to produce irritation than is a dry one.

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December 15, 1949

MN073280

Dr. V. A. Drill
Department of Pharmacology and Therapeutics
Wayne University College of Medicine
1512 St. Antoine Street
Detroit 26, Michigan

Dear Dr. Drill:

We are sending to you under separate cover, samples of 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) for use in the toxicological studies on dogs.

The sample of 2,4-D being sent is regular production material assaying 98.5% and having a freezing point of 132.8°C.

The sample of 2,4,5-T being sent is also regular production material assaying 100% and having a freezing point of 148.8°C.

Both of these materials are being supplied to you in the acid form. Both are fairly soluble in olive oil and both can easily be put in aqueous solution with caustic. I believe you will find that a slight excess of caustic is required to put these materials in solution and that once in solution, a large part of the excess alkali can be neutralized. Hence, for a 5.0% aqueous solution of the sodium salt of 2,4-D, the pH after solution is effected, can be adjusted down to 7.2 with HCl; with 2,4,5-T the final pH of a 1.0% aqueous solution can probably be in the neighborhood of 10.

When 2,4-D or 2,4,5-T is used as an herbicide, the concentration usually ranges from 0.1% to 0.2%. The active material may be applied in any one of a number of forms depending upon the particular requirements of the job. The most common forms are the sodium salt, alkanol amine salts, and esters.

Almost all the toxicological work so far reported deals with 2,4-D administered either as the free acid in oil or as the sodium salt in aqueous solution at a pH of 7.2. We have done a fair amount of toxicological work on the other forms of 2,4-D and also on 2,4,5-T in its various forms. We have observed that the form of the material makes little difference to mammals and that, acutely, there is not much difference between 2,4-D

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The toxicity picture as we have it is given in the following tabulations:

Toxicity of 2,4-D when administered in Single Doses.

Animal	Route	Source	Approx. LD_{50} (g./kg.)
Rat	Oral	Lit. Na Salt	0.666
Rat	Oral	Dow-Incomplete	
		Na Salt	0.70
		Acid	0.5 - 0.6
		Isopropyl esters	0.5 - 0.7
Mouse	Oral	Lit. Na Salt	0.375
		Dow - Incomplete	
		Acid	0.3 - 0.5
Mouse	Sub-Cut.	Lit. Na Salt	0.280
Guinea Pig	Oral	Lit. Na Salt	1.000
		Dow - Incomplete	
		Na Salt	0.5 - 0.7
		Acid	0.5 - 0.7
Chicks	Oral	Lit. Na Salt	0.38 - 0.76
		Dow - Just started	

Toxicity of 2,4-D when administered in Repeated Doses.

Animal	Route	Source	Notes
Dogs	Sub Cut or I.V.	Lit.	Na Salt 0.200 g./kg./day-death in 2 da. 0.100 g./kg./day death in 2-4 days 0.050 g./kg./day death in 3 days 0.025 g./kg./day marked affects
Guinea Pigs	Orally (?)	Lit.	Na Salt 0.100 g./kg./day 10 times in 12 da. was tolerated
Rats	Orally in diet	Lit. Dow	0.1% tolerated 0.1% -caused very slight effects 0.03% clear
Mice	I.P.	Lit.	No marked histopathological findings No neoplastic growths Reproduction O.K.

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Toxicity of 2,4-D when administered in Repeated Doses. - Cont'd

Animal	Route	Source	Notes
Chicks	Orally	Lit.	0.028 g./kg. 3 times/week for 4 weeks - O.K. 0.280 g./kg. 3 times/week for 4 weeks - growth depression
	Orally in Diet	Dow	0.1% for 1 week - O.K. 0.3% for 1 week - O.K. except for slightly depressed growth rate.

Toxicity of 2,4,5-T When Administered in Single Doses Orally

(This information is from our data and is not complete - We are finishing it.)

Animal	Notes
Rat	LD_{50} for the acid lies between 0.5-1.0 g./kg. LD_{50} for the isopropyl ester lies between 0.3 - 1.0 g./kg.
Mouse	LD_{50} for the acid lies between 0.3-0.7 g./kg.
Guinea Pig	LD_{50} for the acid lies between 0.3-1.0 g./kg. For both the acid and Na salt
Chick	Work just begun

Neither 2,4-D nor 2,4,5-T are more than very mild skin irritants even in concentrated form. In diluted form ready for use, they present no health hazard.

Studies conducted upon guinea pigs by Hill and Carlisle on the inhalation of 2,4-dichlorophenoxy acetic acid dust, either wet or dry, but of unknown particle size, indicate that the dust is not likely to cause systemic intoxication. This has been born out by our own experience with men handling the dust. The dust, however, is capable of causing irritation of the nose if the concentration in the breathing zone becomes sufficiently high.

The following references may be helpful if you wish to consult the literature for a more detailed description of procedures, etc.:

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J. Ind. Hyg. Toxicol. 29, 85-95 (1947)
C.A. 41: 3217g (May 20, 1947)

2. Tolerance of Farm Animals to Feed Containing 2,4-Dichloro phenoxy acetic acid.
J. W. Mitchell, R. E. Hodgson and C. F. Gaetjens (U.S. Dept. of Agr., Washington, D. C.)
J. Animal Sci. 5, 226-32 (1946)
C.A. 40 (17); 5198g (Sept. 10, 1946)

3. 2,4-D Toxicity. I. Toxicity Towards Certain Species of Fish.
Jos. W. E. Harrisson and Edward W. Rees (La Wall and Harrisson, Research Consultants, Philadelphia, Pa.)
Am. J. Pharm. 118, 422-5 (1946)
C.A. 41 (2529) (1947)

4. Effects of 2,4-Dichlorophenoxy Acetic Acid on Experimental Animals.
Nancy L. R. Bucher (Harvard University)
Proc. Soc. Exptl. Biol. Med. 63, 204-5(1946)
C.A. 41: 808 (1947)

Data on the acute oral toxicity of both 2,4-D and 2,4,5-T for dogs is lacking. Therefore, it is suggested that the acute oral studies be started at your earliest opportunity so that information will be available for choosing appropriate dosage levels for the 90 day feeding tests.

In the single dose experiments we suggest that a notation be made regarding any symptoms exhibited at the various dosage levels, body weights for each day during 3 or 4 days immediately following administration and on alternate days during the rest of the 2-week observation period. We do not feel that it is necessary to do any elaborate tissue studies on acutely dosed animals. We would, however, like to see animals that die and those that survive autopsied and the gross condition of the G.I. tract, liver, and kidneys noted. A few sections at critical dosage levels might also be desirable - at your discretion.

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file

DOW 7358

In the 90 day feeding tests, we suggest that the material be administered with a part of the diet. Dosage levels cannot be decided upon until acute data is available. We would suggest that general observations, growth records, and periodic hematological examinations (initially, after 30 days, and after 90 days on the experiment) be conducted during the experiment. At autopsy, gross and histopathological studies on the liver, kidney, stomach, intestine, spleen, lung, heart, adrenal, testis or ovary, and possibly brain and vascular system are of importance. We believe that negative findings at upper dosage levels make similar studies at lower dosage levels unnecessary.

The requisition for this work has been approved and I will see that an appropriate check is deposited as before.

If there are any details which are not clear or if any problems arise, please contact us at once.

I regret that I did not have more time last week to spend with you. Nevertheless, I was very glad to get home early and avoid the snowstorm that settled on this area about 5:00 p.m. that evening. Please give my regards to Dr. Hays.

Sincerely yours,

THE DOW CHEMICAL COMPANY

V. K. Rowe

V. K. Rowe
Biochemical Research Department

VKR/ug

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Reference 6

ACUTE ORAL TOXICITY OF 2,4-DICHLORO-PHENOXYACETIC ACID TO RATS, MICE, GUINEA PIGS AND CHICKS

CONTAINS TRADE SECRET OR OTHERWISE CONFIDENTIAL INFORMATION
THE DOW CHEMICAL COMPANY

DOW508334

V. K. Rowe
D. D. McCollister
and
H. C. Spencer

Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan

The 2,4-dichlorophenoxyacetic acid was administered by oral intubation to rats, mice, guinea pigs and chicks as a solution in olive oil emulsified with 5-10% gum arabic solution. For comparison, the sodium salt of 2,4-dichlorophenoxyacetic acid was fed in aqueous solution to rats and guinea pigs.

DOW 5083335

The guinea pigs and the white rats were mature, young adult animals selected from the stock colonies of this laboratory. Young adult white mice were obtained from the Carworth Farms, Rockland, New York. The chicks (New Hampshire Red) were purchased from a commercial hatchery and used when about three weeks of age. Mixed sex groups of each species were used in these tests.

Table I summarizes the concentrations and volumes of olive oil solutions of 2,4-dichlorophenoxyacetic acid administered to each species.

TABLE I

SINGLE ORAL ADMINISTRATION OF
2,4-DICHLOROPHENOXYACETIC ACID

Species	Per cent Material in Olive Oil	Volume Given by Stomach Tube	
		Minimum (cc.)	Maximum (cc.)
Rats	5, 10	0.4	4.0
Guinea Pigs	10	0.9	2.9
Mice	5, 10	0.1	0.22
Chicks	3	0.3	5.2

Mortalities resulting from the various single oral dosages of 2,4-dichlorophenoxyacetic acid and its sodium salt are given in

Tables II and III. All surviving animals were observed until it was certain that they had fully recovered (usually about two weeks). Symptoms of poisoning, particularly with the higher doses, were ataxia and myotonia.

DOW 508336

TABLE II

MORTALITIES RESULTING FROM ADMINISTRATION OF SINGLE ORAL DOSES OF 2,4-DICHLOROPHENOXYACETIC ACID

Dosage (gm./kgm.)	Rats		Guinea Pigs		Mice		Chicks	
	No. Fed	No. Died	No. Fed	No. Died	No. Fed	No. Died	No. Fed	No. Died
0.1	10	0					6	0
0.2	10	0			6	0		
0.3	10	3	6	0	5	1	6	1
0.4	10	6			6	4		
0.5	10	7	6	4	6	6	6	6
0.6	10	10						
0.7	3	3	6	6	6	6	6	4
1.0							6	6

TABLE III

MORTALITIES RESULTING FROM ADMINISTRATION OF SINGLE ORAL DOSES OF 2,4-DICHLOROPHENOXYACETIC ACID, SODIUM SALT

Dosage (gm./kgm.)	Rats		Guinea Pigs	
	No. Fed	No. Died	No. Fed	No. Died
0.3	10	0	6	0
0.5	10	2	6	4
0.7	10	4	6	6
1.0	14	14		

The acute oral LD_{50} values with their 19/20 confidence limits were determined for each according to the method described by Litchfield and Wilcoxon[1]. The results of the statistical treatment of the dosage-response data are presented in Table IV.

5091

1. Litchfield, J. T., Jr. and Wilcoxon, F.: J. Pharm. and Expt. Ther. 96:99. 1949.

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TABLE IV

THE ACUTE ORAL LD_{50} VALUES FOR 2,4-DICHLOROPHENOXYACETIC ACID WHEN ADMINISTERED TO RATS, MICE, GUINEA PIGS AND CHICKS

Species	Form	LD_{50} (19/20 Confidence Limits) gm./kgm.	Slope Function*
Rats	Oil-acid	0.375 (0.302-0.465)	1.62
Mice	Oil-acid	0.368 (0.312-0.434)	1.23
Guinea Pigs	Oil-acid	0.469 (0.397-0.553)	1.17
Chicks	Oil-acid	0.541 (0.358-0.817)	1.81
Rats	Aq.-Na salt	0.805 (0.610-1.063)	1.77
Guinea Pigs	Aq.-Na salt	0.551 (0.417-0.727)	1.55

*The fold change in dosage required to produce one unit standard deviation in response along the line.

DOW5083337

The Litchfield and Wilcoxon test for parallelism of two lines revealed that the dosage-response line for the rats (oil-acid) deviated significantly (19/20 probability) from those for the mice and the guinea pigs receiving the 2,4-dichlorophenoxyacetic acid in olive oil. The line for the chicks also deviated significantly in parallelism from those for the guinea pigs and mice. The tests for parallelism of two lines and estimate of relative potency indicated no statistically significant differences in comparison of rats versus chicks and mice versus guinea pigs.

The dosage-response curves for guinea pigs (oil-acid) and guinea pigs (aqueous-sodium salt) did not deviate significantly in parallelism or relative potency. A comparison of the rats (oil-acid) versus rats (aq.-Na salt) revealed no significant difference in parallelism of the two lines, but the test for relative potency indicated that the free acid was significantly more toxic to rats than was the sodium salt.

10

Reference 7

CONTAINS
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SEMICHRONIC ORAL TOXICITY OF

2,4-DICHLOROPHENOXYACETIC ACID TO RATS

DOW 5083338

I. Oral Administration - Repeated Doses by Stomach Tube.

II. Oral Administration in the Diet

V. K. Rowe
D. D. McCollister
and
H. C. Spencer

Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan

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DOW 508339

I. ORAL ADMINISTRATION - REPEATED DOSES BY STOMACH TUBE

Experimental Procedure

Thirty-one female rats, 60 to 80 days old, were divided according to body weights into groups of five each (six in the control group) and maintained on the stock rat diet. Before the repeated oral administration of 2,4-dichlorophenoxyacetic acid was started, weight records were kept on the animals for a period of 21 days to make certain that the groups were well matched in respect to growth.

The rats in each of the groups were given by means of a stomach tube repeated oral doses of 2,4-dichlorophenoxyacetic acid dissolved in olive oil and emulsified in 2 to 3 cc. of a 5-10 per cent gum arabic solution. The dosages fed and the olive oil solutions used are given in Table I.

TABLE I

DOSAGES ADMINISTERED AND OLIVE OIL SOLUTIONS OF 2,4-DICHLOROPHENOXYACETIC ACID USED

Dose g./kg.	Per cent 2,4-Dichlorophenoxy-acetic acid in Olive Oil	Approximate Vol. of Olive Oil Solution Administered per Rat per Dose
0.0	0.00	1.5
0.003	0.06	1.0
0.01	0.20	1.0
0.03	0.60	1.0
0.1	2.00	1.0
0.3	6.00	1.0

The animals were weighed at intervals of two and three days during the experiment, and a record was kept of the average growth of each group. Hematological examinations were made on the control rats and on those that had received 20 oral doses of 0.03 and 0.01 g./kg. 2,4-dichlorophenoxyacetic acid. At the end of the experiment, all of the surviving rats were killed by decapitation, and oxalated blood was collected for urea-N determinations.(1) Each rat was examined for gross pathological changes, organ (heart, liver, kidneys, spleen) weights were obtained, and tissues were saved for microscopic examination. Hematoxlin and eosin-stained sections of the following organs were prepared: lung, heart, liver, kidney, spleen, adrenal, pancreas, stomach and intestines.

DOW 5083340

EXPERIMENTAL RESULTS

The rats that received 20 oral doses in 28 days of 0.003, 0.01, and 0.03 g./kg. 2,4-dichlorophenoxyacetic acid showed no ill effects so far as could be judged from gross appearance and behavior, survival, growth, hematology (Table II), blood urea-N values (Table III), organ weights (Table III), gross appearance of organs, and histopathology.

(1) Barker, S. B. The Direct Colorimetric Determination of Urea in Blood and Urine. J. Biol. Chem. 152: 453-463, 1944.

TABLE II

AVERAGE HEMATOLOGICAL VALUES OBTAINED ON FEMALE RATS THAT HAD RECEIVED 20 ORAL DOSES IN 27 DAYS OF 2,4-DICHLOROPHENOXYACETIC ACID

Dose, g./kg.	Controls	0.03	0.01
Number of rats in group	6	5	5
Erythrocytes (million per cu.mm.)	11.4	10.5	10.0
Hemoglobin (g./100 cc.)	13.9	13.3	12.9
Leucocytes (thousand per cu.mm.)	18.8	18.3	17.4
Differential Count:			
Neutrophils (per cent)	10	15	9
Lymphocytes (per cent)	90	85	91

TABLE III

AVERAGE BODY WEIGHTS, ORGAN WEIGHTS AND BLOOD UREA-N VALUES OF FEMALE RATS THAT RECEIVED REPEATED ORAL DOSES OF 2,4-DICHLOROPHENOXYACETIC ACID

Dose g./kg.	No. of Doses	No. of Days	No. of Rats Surviving	Body Wt. (g)		Organ Weights (g./100 g.)				Blood Urea-N mg. %
				Initial	Final*	Heart	Liver	Kidney	Spleen	
0.00	0	28	6	195	203	0.42	3.40	0.75	0.23	24.9
0.003	20	28	5	191	197	0.40	3.37	0.75	0.22	24.8
0.01	20	28	5	191	195	0.42	3.38	0.79	0.19	32.8
0.03	20	28	5	187	192	0.41	3.25	0.81	0.23	25.9
0.1	15	21	3	190	126	0.51	4.68	0.94	0.16	26.6
0.1	15	21	2	194	202	0.43	4.08	0.87	0.23	17.2
0.3	2	2	2	195	186	0.48	3.08	0.82	0.17	--

*Fasted overnight.

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DOW 508341

Of the five rats that received repeated oral doses of 0.1 g./kg. 2,4-dichlorophenoxyacetic acid, three lost weight rapidly after the first few doses and showed a marked diarrhea. On the other hand, the other two rats receiving 0.1 g./kg. maintained their initial body weights and showed no evident ill effects so far as general appearance and behavior were concerned. All of these rats were killed and autopsied after they had received fifteen doses in a period of twenty-one days. The outstanding finding in these animals was varying degrees of gastrointestinal irritation; there was also slight cloudy swelling of the liver with an increase in weight of this organ (Table III). Blood urea-N values obtained on these animals compared favorably with those for the controls (Table III).

DOW 508342

Three of the five rats fed 0.3 g./kg. 2,4-dichlorophenoxyacetic acid died after the second dose. Consequently, the two surviving animals were killed and examined; severe gastrointestinal irritation was observed in both of these rats.

SUMMARY AND CONCLUSIONS

Female rats that received 20 oral doses in 28 days of 0.003, 0.01, and 0.03 g./kg. 2,4-dichlorophenoxyacetic acid showed no adverse effects as judged by gross appearance and behavior, mortality, growth, hematological values, blood urea-N determinations, organ weights, and gross and microscopic examination of the tissues.

On the other hand, rats receiving repeated oral doses of 0.1 g./kg. 2,4-dichlorophenoxyacetic acid showed varying degrees of gastrointestinal irritation with marked weight loss in three out of five animals. Higher doses (0.3 g./kg.) were not tolerated upon repeated administration.

DOW 5083343

II. ORAL ADMINISTRATION IN THE DIET

Experimental Procedure

The modified Sherman diet, which has been used successfully for several years in this laboratory as the stock ration for rats, served as the control and basic diet in this experiment. The following ingredients were used in the preparation of this stock diet:

	Percent by weight
Whole wheat, freshly ground	55
Dried whole milk	25
Dried extracted liver	12
Dried yeast	5
Iodized table salt	2
Calcium carbonate	1

The experimental diets were prepared by thoroughly mixing finely ground 2,4-dichlorophenoxyacetic acid with the stock diet on a "percent by weight" basis. No diet preparations over a month old were used.

Female white rats from the stock colony of this laboratory were used in this investigation. These animals were the descendants of rats obtained in 1938 from the Wistar Institute. Five rats were caged together in wire bottom cages. The animals were fed from aluminum hoppers which were weighed and refilled three times a week. Each rat had access to food and water at all times.

The rats were maintained on the stock diet from the time of weaning until they were 2 to 3 months of age, when they were divided according to body weights into well matched groups and started on the experimental diets. Groups of 5 female rats each were placed on diets containing 0.00 (control), 0.01, 0.03, 0.10, 0.30 and 1.00 percent 2,4-dichlorophenoxyacetic acid.

All rats were weighed twice a week throughout the course of the experiment. Records were kept of body weight, general appearance, and estimated average daily food consumption of each animal.

During the course of the experiment, rats that were obviously quite sick were killed and examined to determine the cause of illness.

At the end of the experimental periods, all surviving rats were fasted overnight, weighed, killed by decapitation and examined. Certain organs (liver, kidneys, heart and spleen) of each rat were weighed and the tissues of all animals of each group were saved for histopathological studies. Hematoxylin and eosin stained sections of the following organs were prepared: lung, heart, liver, kidney, spleen, adrenal and pancreas. In addition, stained sections were prepared from the stomachs of the rats that received the diets containing 1.00 and 0.30 percent of the test material.

The concentration of urea-nitrogen of the blood was determined by the diacetyl monoxime method[1] on the control rats and on those that had been maintained for 12 days on diets containing 1.00 and 0.30 percent 2,4-dichlorophenoxyacetic acid and 113 days on diets containing 0.10, 0.03 and 0.01 percent of the test material.

1. Barker, S. B., J. Biol. Chem. 152: 453-463, 1944.

DOW 508344

Experimental Results

DOW 506345

Food Consumption

The daily food intake of the rats fed the 0.10, 0.03 and 0.01 percent diets was found to be from 10 to 15 grams per rat. It may be calculated that these rats, weighing about 200 grams, ingested quantities of 2,4-dichlorophenoxyacetic acid of the order of 0.06, 0.02 and 0.006 gm./kgm./day, respectively. On the other hand, food consumption of the rats fed the 1.00 and 0.30 per cent diets was quite low, the exact amount eaten being uncertain because the animals wasted a great deal by pawing and scratching at the hoppers.

Growth and Survival

The rats on diets containing 1.00 and 0.30 percent 2,4-dichlorophenoxyacetic acid lost weight rapidly after the start of the experiment (see chart) so that they were killed and examined after 12 days. One rat on the 0.30 percent diet died (Table IV).

The rats receiving the diet containing 0.10 percent 2,4-dichlorophenoxyacetic acid did not grow well. Only one rat survived the full experimental period (113 days). Two of the rats in this group were killed because of spontaneously occurring diseases (pneumonia and ear infection), one was missing from the cage, and the fourth was found dead in the cage in such a condition that the cause of death could not be ascertained.

The rats in the groups receiving 0.03 and 0.01 percent 2,4-dichlorophenoxyacetic acid in the diet grew as well as the animals receiving the control ration. Deaths in these groups were attributable to either pneumonia or ear infection. Hematological values obtained on the blood of these rats after 106 days on the diets as well as those of the control group are summarized in Table V. All results were within normal limits.

TABLE IV

SURVIVAL OF FEMALE RATS ON DIETS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID

Days on Experiment	Number of Rats Surviving on Each Dietary Level					
	0.00	0.01	0.03	0.10	0.30	1.00
0	5	5	5	5	5	5
9	5	5	5	5	4[a]	5
12	5	5	5	5	4[b]	5[b]
20	5	5	5	5		
30	5	5	4[c]	5		
41	5	5	4	4[a]		
51	4[d]	5	4	4[e]		
62	4	5	4	3		
69	4	5	3[d]	3		
79.	4	5	3	2[d]		
90	4	4[d]	3	2		
100	4	4	3	2		
113	4	4	2[c]	1[c]		

a- dead in cage

b- animals losing weight, sacrificed for histopathological examination

c- pneumonia

d- ear infection

e- missing from cage

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General Appearance

The rats killed after 12 days on the 1.00 and 0.30 percent diets appeared thin and unkempt. Fat depots were depleted. On the other hand, the rats maintained for 113 days on the 0.10, 0.03 and 0.01 percent diets appeared to be in good condition.

Body and Organ Weights

A summary of the average final body weights, organ weights and blood urea-nitrogen is given in Table VI. Increased organ weights on the "grams per hundred grams" basis for the groups of rats receiving the 1.00, 0.30 and 0.10 percent diets as compared with the controls was due entirely to decreased body weights. The body weights and organ (heart, liver, kidneys, spleen) weights of the rats on the 0.03 and 0.01 percent diets were normal.

TABLE V

HEMATOLOGICAL VALUES FROM FEMALE RATS MAINTAINED FOR 106 DAYS ON DIETS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID

Rat Number	Percent 2,4-D	Erythrocytes (million/ cu.mm.)	Hemoglobin (gm./100cc.)	Leucocytes (thousand/ cu.mm.)	Diff.Count(percent) Neutr.	Lymph.	Eosin.
8092	0.0	9.0	14.4	14.0	29	68	3
8076	0.0	9.3	14.2	21.7	14	78	8
7950	0.0	8.1	14.0	13.5	-	-	-
8099	0.0	8.4	14.1	13.4	20	71	9
7947	0.1	9.2	14.0	22.6	25	72	3
8079	0.1	9.9	14.7	20.5	-	-	-
8101	0.03	9.0	15.1	18.7	12	83	5
8088	0.03	9.0	13.6	24.0	12	82	6

TABLE VI

AVERAGE BODY WEIGHTS, ORGAN WEIGHTS AND BLOOD UREA-NITROGEN VALUES FROM GROUPS OF FEMALE RATS MAINTAINED ON DIETS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID

Percent in Diet	Number of Rats	Days on Diet	Body Wt. (gm_a) Initial	Body Wt. (gm_a) Final	Organ Weights (gm./100 gm. body wt.) Heart	Liver	Kidneys	Spleen	Blood Urea Nitrogen (mgm.%)
0.00	4	113	174	206	0.40	2.44	0.73	0.33	29.2
0.01	4	113	175	207	0.39	2.33	0.72	0.30	18.9
0.03	2	113	182	203	0.36	2.39	0.75	0.25	17.1
0.10	1	113	176	175	0.38	3.14	0.78	0.34	12.5
0.30	4	12	177	145	0.41	3.57	0.89	0.39	37.7
1.00	5	12	175	127	0.39	4.72	0.85	0.47	20.1

a Fasted overnight

DOW 5083348

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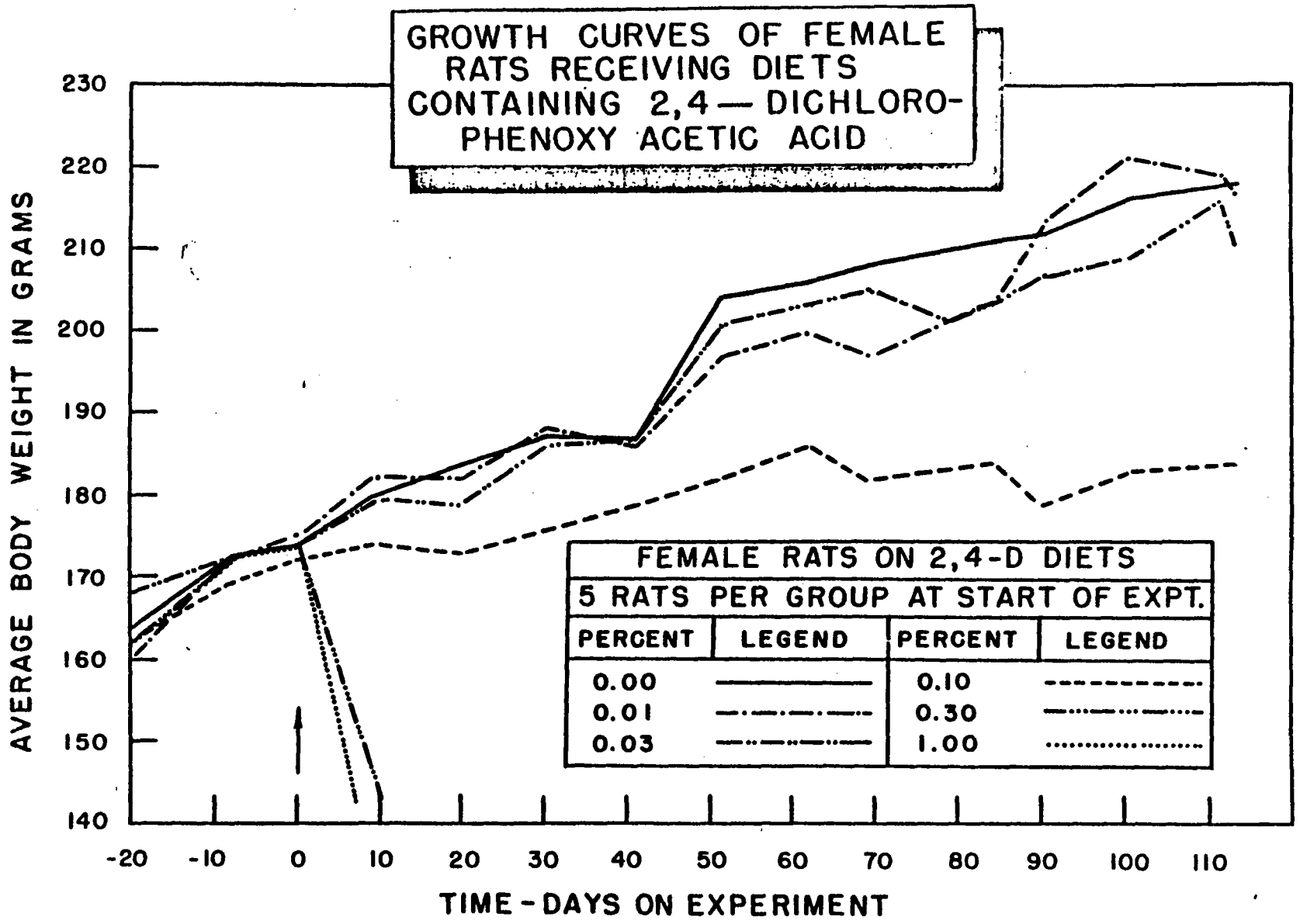
DOW 508349

Histopathology

Microscopic examination of the tissues revealed a slight parenchymatous degeneration of the liver cells in the rats on the 1.00 percent diet. Two of the four rats on the 0.30 percent diet showed slight cloudy swelling in the liver and in the proximal convoluted tubules of the kidneys. There was no evident irritation of the stomach mucosa of the rats on the 1.00 and 0.30 percent dietary levels. The one rat surviving one the 0.10 percent diet showed only a very slight cloudy swelling in the liver. None of the animals on the dietary levels of 0.03 and 0.01 percent 2,4-dichlorophenoxyacetic acid showed any changes upon histopathological examination.

SUMMARY AND CONCLUSIONS

Groups of 5 female rats each maintained for 113 days on diets containing 0.00, 0.01 and 0.03 percent (0, 100, and 300 p.p.m.) 2,4-dichlorophenoxyacetic acid showed no adverse effects as judged by growth, food consumption, general appearance, mortality, blood urea-nitrogen concentrations, hematological examination, organ weights, and gross and microscopic examination of the tissues. On the other hand, slight adverse effects were observed at concentrations of 0.10 percent (1000 p.p.m.) fed in the diet for 133 days. Extreme weight loss of the rats on the 0.30 and 1.0 percent (3000 and 10,000 p.p.m.) diets was associated with a low food intake and necessitated sacrificing the animals on these dietary levels 12 days after feeding was begun.



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2,4,5-T ACID

Trichlorophenoxy Acetic Acid	98.93%
Trichlorphenol	0.76%
Sodium Chloride	0.6%
Freeze Point	150.6°C.
Color	White

2,4-D ACID Batch 7-2 (new acid)

		old acid.
2,4-D	99.35%	98.8
Dichlorophenol	0.18%	0.277
Sodium Chloride	0.035%	0.08
Freeze Point	136.5°C.	135.6
Color	White	

L. C. White

L. C. White
Britton's Division
2-267 Building

LCW/rj
4/11/50

11A

Biochemical Research Department
THE DOW CHEMICAL COMPANY

File T23.14-23-4
Chg. 5862
Rec'd 12-16-49
Fin'd 8-30-50
Work By Dr.V.A.Drill
Wayne Univ.

Subject: INTERIM REPORT ON THE ACUTE AND SUBACUTE ORAL TOXICITY OF 2,4,5-TRICHLOROPHENOXY-ACETIC ACID FOR DOGS.

To J. W. Britton
Executive Research Committee

Check H. Gilmer 9-5-50

Rept. By V. A. Drill 8-31-50

DOW MN073280 758852

UNIT INDEX

A copy of a report of the acute oral studies and a progress report of the 3 month studies being carried out by Dr. V. A. Drill of Wayne University on 2,4,5-trichlorophenoxyacetic acid is given.

INDEX HEADINGS

2,4,5-Trichlorophenoxyacetic acid

Acetic Acid: 2,4,5-trichlorophenoxy-,

Phenoxyacetic acid: 2,4,5-trichloro-,

MATERIAL

The material used in this study was a sample designated as K4568-5. It was supplied by L. C. White and bore the reference number 20W-108, Batch 138, Sample 6. The freezing point was 150.6°C and an assay showed the material to be 98.93% pure.

PROBLEM

In order to have some toxicological information relative to the effects of 2,4,5-T when fed repeatedly in single doses to a larger laboratory animal, the dog, a project was set up with Dr. Drill at Wayne University. A progress report of this work which was filed as an exhibit in evidence at the F.D.A. Hearing for the establishment of residue tolerances on fresh fruits and vegetables comprises a part of this report.

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UCW 75853

CONCLUSIONS

The acute toxicity of 2,4,5-T for dogs is somewhat greater than for rats. In general, however, 2,4,5-T resembles 2,4-D in toxicity.

1a (5)

C6048[illegible]

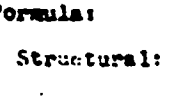
12

DOW 2239339

IDENTITY

Name: 2,4-Dichlorophenoxyacetic acid

Formula:

Structural: 

Empirical: $C_8H_6Cl_2O_3$

B.R.D. K No.: 2372-16

Material received from A. E. Colby, 207 Building, 5-31-49

Ref. No.: 16713

CONCLUSIONS

It appears from the data that we have obtained and also from that which appears in the literature that 2,4-D is moderately toxic and although it would be possible for a person to consume a sufficient amount of material to be dangerous to life, this does not seem likely except possibly where concentrated solutions are being handled and are available to children or livestock. It does not seem likely that dilute solutions such as are used in ordinary spray techniques in the field could present any serious ingestion hazard.

5114 0001733

K No. 4568-S

Biochem. File T2314-23-4

REQUEST FOR BIOLOGICAL TEST
TO BIOCHEMICAL RESEARCH LABORATORY

C.R.I., File Name:

2,4,5-Trichlorophenoxyacetic Acid.

Structural Formula: Molecular Formula

DOW 7588854

RECEIVED DEC 19 1949

Constituents in Copolymers or Mixtures:

2,4,5-T	98.93%	
Trich ϕ	0.76%	Date
Na Cl	0.60	

Suggested Solvents: Discussed with

Requested by VKK 12-16-49

Made by L. C. White 2-24-49

Reference 20-W-108 Amt. Sent

Batch 138 Sample 6

P.P 153.6 °C BP °C at mm. Hg Dens. at °C/ °C

Handling Precautions

Product State: Research Development Spec. Chem. Plant

Use contemplated

Tests Requested: Toxicity of — for dogs.
acute + 3-month oral by diet.
— VKK 12-16-49.

Charge No. 5862

Lit. Ref. Discl. No. Patent

(Please do not write below this line)

Tests	By	Date
	CC04837	
General screening 98% 12-22-49		

5112

Sample to

Noted By D.W.G. Date 12/27/49 By Date

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Désherbants sélectifs et substances de croissance.

Aperçu technique.
Effet pathologique sur l'homme au cours de la fabrication de l'ester du 2.4 D. (1)

Par M.

M. ASSOULY

(Paris).

Le désir d'obtenir de belles récoltes a, de tous temps, été le souci permanent des agriculteurs. Pour y parvenir, ils ont essayé divers moyens : enrichissement du sol par des engrais; accroissement du rendement par l'emploi de substances de croissance; enfin, destruction des mauvaises herbes qui, en absorbant les engrais, l'eau et les matières minérales, entravent le développement des plantes utiles.

On est parvenu aujourd'hui, dans le domaine des produits de croissance et des herbicides, à des résultats tout-à-fait remarquables. Mais leur fabrication suscite des inquiétudes dont le *Conseil Supérieur d'Hygiène de France* s'est fait l'écho dans sa séance du 13 février dernier. On a d'abord pensé que ces produits pouvaient présenter une action cancérigène, et cette appréhension s'explique fort bien quand on voit la manière dont ces substances agissent sur les plantes, où elles provoquent des tumeurs, des hyperplasies. Mais finalement, cette action cancérigène a été écartée par le professeur Simonnet. Par contre, le *Conseil Supérieur d'Hygiène de France* a souligné le danger d'intoxication que pouvaient présenter ces produits pour les ouvriers qui les fabriquent.

Avant de nous attacher à l'étude de la toxicité des substances en cause, nous croyons utile de rappeler quelques notions techniques à leur sujet.

COMPOSITION ET ACTION DES HORMONES VÉGÉTALES

Nous avons employé les mots d'hormones végétales, parce que c'est ainsi que l'on désigne couramment ces produits destinés à accroître le rendement. Il conviendrait peut-être d'employer plutôt (comme le remarque Pierre Chouard) le nom de *substances de croissance* qui désigne, sans préjuger de leur nature intime, les agents chimiques de tous ordres, capables d'exercer, à de très petites doses, une stimulation puissante sur l'élongation des cellules végétales jeunes. Primitivement, ces substances ont été appelées *auxines* ou *hormones de croissance végétales* ou *phyto-hormones*.

En réalité, les *auxines* sont seulement les substances fabriquées par la plante elle-même et qui exercent ce phénomène de stimulation, d'élongation cellulaire par un mécanisme hormonal.

On s'est aperçu, ces 10 dernières années, qu'une multitude d'autres substances avaient le même pouvoir que les auxines; on les a dénommées *hormones végétales*.

Ces hormones ont une action qui varie suivant le moment d'utilisation et le but recherché : elles exaltent la croissance des plantes ou, au contraire, la ralentissent; elles accélèrent la germination des graines, excitent la formation de racines, de germes, de boutons, de fleurs; ou, au contraire, ralentissent ces fonctions; elles permettent la formation artificielle de fruits sans graines, sans fécondation par le pollen; elles aident

(1) Communication à la *Société de Médecine du Travail*, le 20 novembre 1950.

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à la réparation des dégâts causés par la gelée, facilitent la transplantation, le bouturage C'est dire que leur champ d'application est vaste.

Que sont ces hormones ? — Ce sont des dérivés oxy-acétiques du noyau benzène et du noyau naphtalène; des acides phényl, naphtyl et indol-acétique, propionique et butyrique, ainsi que certains de leurs dérivés, comme par exemple des nitriles, des esters alkyliques, des sels d'alcali et d'ammonium, etc... L'un des plus connus est l'acide 2-4 dichlorophénoxyacétique, qu'on appelle, par abréviation, le 2-4 D.

Chacun de ces produits a une action particulière et leur emploi varie donc suivant le résultat à obtenir : par exemple, pour le bouturage, on utilise l'acide indol-acétique, l'acide indol-butyrique ou l'acide naphtalène-acétique plutôt que le 2-4 D. qui a une action trop déformante dans ce cas; contre la chute des fruits, on se sert de l'acide naphtalène-acétique ou de ses sels, ou bien encore du 2-4 D. mais à grande dilution. Signalons aussi que le 2-4 D. appliqué en pulvérisations ou aérosols, stimule puissamment la maturation des fruits.

Un nombre considérable de corps ont été ainsi étudiés et mis au point et certains (comme le 2-4 D. dont nous parlons plus haut) se sont révélés être de l'ordre d'une centaine de fois plus actifs que l'auxine proprement dite; si bien qu'aux doses ordinaires ce n'est plus le pouvoir normal de croissance qui s'exerce, mais l'excès de ce pouvoir, aboutissant à la toxicité. De cette toxicité a dérivé un emploi nouveau : l'utilisation comme herbicide.

Cette utilisation des hormones comme herbicides sélectifs est venue aussi de la connaissance de leur action stimulante sur les diverses fonctions de la plante, y compris la formation de tumeurs. L'usage des hormones, en tant que substances de croissance, provoque souvent, après l'application, des hyperplasies, des cals cicatriciels qui se forment sur la surface de section d'un organe, tige ou pétiole en général. Les hormones, à doses herbicides, ont une action intensifiée; elles provoquent une exaltation folle des manifestations de croissance par hypertrophie et hyperplasie, une activité désordonnée qui entraîne l'apparition de monstruosités les plus diverses, la consommation intense des réserves et finalement la mort, au stade herbacé.

Il s'agit donc d'une destruction radicale, puisqu'il n'y a pas formation de graines.

Cette action est, en outre, sélective; les hormones s'attaquent aux mauvaises herbes mais respectent les plantes utiles. Et ceci s'explique facilement : la résistance des plantes varie suivant les espèces; celle des céréales, par exemple, est beaucoup plus forte que celle des ravenelles, coquelicots ou bleuets. C'est pourquoi le traitement est sans effet sur les céréales, à condition cependant qu'elles aient dépassé le stade 3 feuilles. Il devient aisé de composer un produit de telle manière que seules les mauvaises herbes soient touchées, tandis que les autres plantes resteront insensibles à son action.

Il convient, en outre, d'observer, lors de l'emploi, certaines précautions pour obtenir un bon résultat. En général, on opère à la fin du printemps, lorsque les tiges des plantes ont atteint leur plein développement et ont épuisé les réserves de leurs racines. On vaporise le produit, en solution, sur le feuillage; il adhère fortement, pénètre rapidement dans les feuilles; la sève le véhicule jusqu'aux racines; il provoque alors, en même temps qu'un arrêt de croissance au stade herbacé, une prolifération désordonnée des cellules qui aboutit à la mort, ainsi que nous l'avons vu. La destruction est obtenue en 15 jours ou 1 mois.

Ces produits présentent un avantage considérable sur les désherbants habituellement employés : chlorate de soude, qui détruit toute végétation sans distinction; acide sulfurique, qui acidifie le terrain, ou sulfate de cuivre, susceptible d'une légère sélectivité, mais dont la manipulation présente de graves dangers ou des inconvénients et dont l'action n'est pas aussi totale.

FABRICATION DE L'ESTER DU 2-4 D

La fabrication de ces hormones végétales s'effectue seulement pendant quelques mois, en général de février à mai, c'est-à-dire pendant la période où l'agriculteur a besoin de ces produits.

Il existe sur le marché deux sortes de produits désherbants; l'un est à base d'ester de l'acide 2-4 D. que l'on émulsionne dans de l'huile minérale avec un mouillant. L'utilisateur procède à une seconde émulsion du produit dans l'eau et le projette ensuite

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sur les surfaces à désherber au moyen d'un pulvérisateur. En cas de pluie, le produit, fixé par le mouillant, adhère néanmoins. Le second qui semble avoir la faveur des agriculteurs, ne contient ni émulsifiant, ni mouillant. A base de sel de soude du 2-4 D. il est directement soluble dans l'eau. Mais il risque d'être lavé par la pluie, puisqu'il ne contient pas de mouillant.

De nouveaux produits à base d'ester sont actuellement en fabrication, comprenant une plus grande quantité d'émulsifiants. On pourra alors verser le produit directement dans l'eau. Le mélange se fera seul, sans qu'on ait même besoin de l'agiter.

Dans l'usine que nous avons visitée, on procédait seulement à la réaction d'estérification de l'acide 2-4 D. qui arrive tout prêt d'une usine de province.

Ouvrons une parenthèse pour signaler que dans cette usine qui fabrique l'acide lui-même, les ouvriers sont imprégnés d'une très forte odeur de phénol, caractéristique de ce produit. Cette odeur est extrêmement tenace; elle persiste, même lorsqu'ils quittent leurs vêtements de travail. Ce qui leur a occasionné quelques désagréments : on considère, par exemple, leur présence indésirable dans les lieux publics, cafés, tramways, etc.... Fermons ici notre parenthèse et revenons à notre usine parisienne.

La réaction d'estérification a lieu à partir du sel de soude de l'acide 2-4 D. sur lequel on fait agir de l'alcool éthylique à 95°, porté à une température de 70°. A ce mélange, on ajoute ensuite de l'acide sulfurique à 98°, ce qui entraîne une élévation considérable de température. La cuve est alors refroidie et maintenue à 70°. Cette opération ayant lieu en vase clos, l'alcool distille et retombe dans la cuve.

Le produit est ensuite lavé à l'eau, toujours en vase clos et on procède à une décantation. Il reste alors dans la cuve un liquide sirupeux qui contient environ 1 p. 100 d'alcool et qui dégage encore une forte odeur de phénol.

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PATHOLOGIE

L'interrogatoire des ouvriers affectés à cette opération révèle qu'ils présentent les troubles suivants : somnolence avec sensation de jambes lourdes, irritation des voies aériennes supérieures, gastralgie avec perte d'appétit, goût sucré dans la bouche avec hypersalivation, sensation d'ébriété et d'hypersensibilité de l'ouïe, le moindre bruit faisant sursauter les ouvriers.

Les ouvriers employés au conditionnement se plaignent d'asthénie avec jambes lourdes, sensation d'ébriété et de tête vide.

A quoi pourraient être dus les symptômes signalés ci-dessus ? On peut imaginer qu'ils sont causés par le produit fini lui-même ou par les émanations des différents constituants, au cours de la fabrication.

C'est ainsi que l'irritation des voies aériennes supérieures est bien connue dans l'intoxication par les phénols, qui produit des catarrhes (laryngites, trachéites).

D'autre part, il est signalé par les auteurs que l'intoxication chronique par le phénol — d'ailleurs discutée — peut se manifester entre autres par des troubles digestifs, anorexie, ptyalisme, céphalées, vertiges. Or, les ouvriers nous ont signalé tous ces signes, parmi les troubles qu'ils rattachent à l'exercice de leur profession.

On pourrait rattacher également quelques-uns des symptômes relevés aux émanations probables des vapeurs d'alcool en cours de fabrication. Cette opinion n'est toutefois pas partagée par l'ingénieur que nous avons vu pendant notre visite, car, d'après lui, d'autres fabrications mettant en jeu des quantités importantes d'alcool ne présenteraient pas les inconvénients signalés par le personnel.

En résumé, on peut donc dire que la plupart des signes pathologiques peuvent être dus au dégagement de vapeurs de phénol. Toutefois, des signes plus particuliers, tels que le goût sucré, la sensation d'hypersensibilité de l'ouïe paraissent originaux et dus au produit fini lui-même.

Les troubles rapportés ci-dessus pourraient constituer un syndrome caractéristique du travail au contact des produits en question, si d'autres auteurs les avaient signalés. Nous avons procédé à des recherches bibliographiques, mais nous n'avons pu trouver de référence, du moins dans les sources que nous avons utilisées, concernant leur action sur le personnel employé à la fabrication.

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Par contre, diverses publications signalent les travaux effectués sur leur toxicologie. Il nous paraît intéressant de résumer ci-dessous les recherches effectuées dans les différents pays du monde.

TOXICOLOGIE

Le professeur R. Fabre, dans un rapport présenté aux Journées Médicales Internationales de Paris en 1937, signale les tumeurs qui se forment chez la plante autour d'une piqûre d'hétéro-auxine, acide indol 3-acétique. Il termine son rapport sur quelques questions pleines d'intérêt : « Les auxines végétales ont-elles une action sur le métabolisme ou la croissance d'un organisme animal ? Aucune expérience définitive n'a été tentée dans ce sens jusqu'ici. Mais il serait fort intéressant d'établir un parallèle entre l'action encore bien discutée des hormones sexuelles sur le développement des plantes et celle des auxines sur le développement des animaux. Et s'il y a action, sur quelle cellule de l'organisme porte-t-elle ? Autant de question sans réponses, qui doivent tenter physiologistes et médecins. »

De même, cette question de la toxicité du 2-4 D. pour les humains fut posée lors de la deuxième réunion du North Central States Weed Control Conference. Quelques membres relatèrent alors des cas d'ingestions accidentelles de quantités variables, mais toujours sans altération apparente de la santé des accidentés. Kraus fit savoir à l'assemblée qu'il avait lui-même absorbé quotidiennement 1/2 g de 2-4 D. pur pendant 3 semaines, sans ressentir de désagrément.

Des expériences furent tentées à Beltsville, afin d'éprouver la toxicité du 2-4 D. sur les animaux. La première consista à faire paître, pendant 15 jours, des vaches et des moutons dans un pâturage préalablement traité avec l'herbicide. On avait même doublé la dose habituellement nécessaire à la destruction des mauvaises herbes. Les animaux furent ensuite examinés par des vétérinaires, et trouvés en excellente santé. Dans la seconde expérience, on fit absorber à une vache 5,5 g de 2-4 D. par jour, et ce pendant 3 mois. L'animal consomma facilement le grain d'alimentation avec lequel la substance chimique était mélangée. Sa production laitière ne subit aucune baisse et un veau, nourri entièrement de son lait pendant 1 mois, ne montra aucun symptôme de maladie. Une autopsie n'a pas révélé d'effet apparent du 2-4 D. sur les différents organes de cette vache.

M. K. Bjorn et H. T. Northen donnèrent pendant 4 semaines une dose quotidienne de 200 mg/kg à des poulets. Cela fut sans effet. Mais la dose de 765 mg/kg fut mortelle.

E. V. Hill, et H. Carlisle, entreprirent des expériences sur 450 souris, 150 rats blancs, 125 cobayes, 70 lapins et 3 singes. Le 2-4 D. fut administré avec les aliments, en injections intra-veineuses, à des doses croissantes pour atteindre et déterminer la dose mortelle. A ce stade, les animaux meurent soudainement de fibrilation ventriculaire aiguë. S'ils échappent à la mort instantanée, on voit apparaître la raideur des extrémités, avec de l'incoordination musculaire, la léthargie, la stupeur et finalement, le coma et la mort.

Ces symptômes sont constants, quelle que soit la façon d'administrer le 2-4 D. et quel que soit l'animal utilisé pour l'expérience.

L'investigation montre que le 2-4 D. est un composé relativement peu toxique dont la dose mortelle est de :

375	mg/kg	de souris,
1 000	—	de cobaye,
666	—	de rat,
800	—	de lapin,

quand ces doses sont administrées en solution aqueuse par la bouche.

En supposant que la tolérance pour l'homme soit similaire à celle des animaux, la plus forte dose que pourrait supporter un homme de 75 kg serait de 15 g.

Ces expériences ont été conduites avec du 2-4 D. utilisé comme herbicide, sans que sa toxicité soit augmentée par celle de ses solvants.

Aucune expérience n'a été entreprise pour mettre en évidence l'intoxication chronique avec les effets de l'absorption par la voie respiratoire.

De leur côté, King et Penfound firent des essais en aquarium sur la brème et le bar. Ils trouvèrent que dans ce milieu clos, le 2-4 D. à 1 p. 100 n'est pas toxique pour ces

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poissons, mais qu'il le devient légèrement à 100 p. 100, concentration qui ne peut être que temporaire et locale en eau courante.

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En vérité, on se trouve là devant un produit nouveau dont le mode d'action lui-même sur les végétaux n'est pas encore très défini en agronomie et reste encore un peu mystérieux.

En ce qui concerne l'organisme humain, le problème est entier, autant du moins que nos recherches nous permettent de l'affirmer.

RÉSUMÉ

On a donné le nom d'hormones végétales à certaines substances chimiques capables d'exercer, à de très petites doses, une stimulation puissante sur l'élongation des cellules végétales jeunes, action exercée habituellement par une substance élaborée par la plante elle-même. Ces hormones sont des dérivés oxy-acétiques du noyau benzène et du noyau naphtalène, des acides phényl, naphtyl et indol-acétique, propionique et butyrique, ainsi que certains de leurs dérivés : nitriles, esters alkyliques, sels d'alcali, d'ammonium, etc... L'un des plus connus est l'acide 2-4 dichlorophénoxyacétique, appelé couramment 2-4 D.

L'action intensive de ces hormones les a fait utiliser comme herbicides sélectifs. Elles agissent en provoquant une exaltation folle des manifestations de croissance, une activité désordonnée qui entraîne l'apparition des monstruosités les plus diverses, la consommation intense des réserves, et finalement la mort au stade herbacé.

Les ouvriers employés à la fabrication de l'ester du 2-4 D. se plaignent de somnolence avec sensation de jambes lourdes, d'irritation des voies aériennes supérieures, de gastralgie avec perte d'appétit, de goût sucré dans la bouche avec hypersalivation, de sensation d'ébriété et d'hypersensibilité de l'ouïe, le moindre bruit les faisant sursauter.

Des expériences faites sur les animaux ont montré que le 2-4 D. absorbé avec les aliments ou inoculé en injections intra-veineuses, n'était toxique qu'à doses assez élevées.

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T-CN PHENOXYACETIC ACID, 2,4-DICHLORO

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Selective Herbicides and Growth Substances. Pathologic Effects on Man During the Manufacture of the Ester of 2,4-D. M. Assouly. Arch. Mal. Profess. 12, 26-30, (1951). (French).

Workers employed in the manufacture of an ester of 2,4-dichlor phenoxyacetic acid complain of somnolescence with heaviness of the legs, irritation of the upper respiratory passages, gastralgia with loss of appetite, of a sweet taste in the mouth with increased salivation, a sensation of drunkenness, and hypersensitivity of hearing, the least sound causing them to jump. In animal experimentation it was shown that when 2,4-D was ingested or injected intravenously, high doses were required to produce intoxication.

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Indirect Effects of Herbicides

C. J. Willard

Under "Indirect Effects of Herbicides" we have included all effects other than direct damage to the weeds or crop plants to which they are applied. The discussion will concern the newer organic herbicides only. This is an extensive litrerature on the effects of sodium chlorate and arsenicals, which does not need reviewing here.

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The indirect effects we have considered are:

1. Effects outside the treated field due to drift or vapors of the herbicides.
2. Unforseen effects on the "balance of nature".
3. Destruction of wild flowers, game cover, etc., in roadside spraying.
4. Poisonous effects of herbicides.
   a. Direct effects
   b. Poisoning from poisonous plants, not usually eaten, but eaten after spraying.
   c. Poisoning from ordinarily harmless plants made poisonous by the effect of sprays on them.
5. Effects on the composition of crops.

The first problem, drift of dust, droplets, or vapors to adjacent or not-so-adjacent susceptible plants, has been so widely discussed that I will take time to make just one point about vapors. Any effect of vapors must be more or less a mass action effect. Dr. Alban and I have used experimental amounts of esters around susceptible plants for years, with no effects on them. But those were small plots. If you spray 10 acres with esters and wind moves slowly across this area (Have you noticed how almost every story of vapor damage begins "There was hardly any wind"?) the air may easily pick up a toxic load of vapor.

As weed control men, we must constantly emphasize the hazards involved in using these extremely powerful chemicals, and personally and by precept, see to it that every precaution is taken against damage where no damage is intended. This cannot be said too often, or made too emphatic. Carelessness in application has already resulted in some severely restrictive laws, and others will follow if they seem to be needed.

Several articles have appeared expressing the fear that we "weed men" will run wild and exterminate local flora of all kinds in all places. Killing plants even with modern chemicals, still takes time and material, which cost money. We are not likely to use either when we do not have an economic reason for doing so. In so doing we may kill some plants that we do not intend to kill, simply because they are in bad company.

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We should not be, nor [illegible] or oblivious to changes in the balance of nature. We should study these changes and, so far as possible, avoid recommendations that produce unfavorable changes, or suggest ways of overcoming them. For example, repeated roadside spraying may take out legumes and, on many soils, require nitrogen fertilization to maintain a healthy sod.

The complaint about killing wild flowers along roadsides is one to which we must, at least partially, plead guilty, but without apologies. Some of these complaints remind me of the anti-vivisection groups throughout the country, to whom, if one were to judge by either actions, the life of a stray dog is more sacred than the lives of children. We will always have some unreasonable complaints. So far as in us lies, we should be "wise as serpents and harmless as doves". We should listen to these complaints, study them, and then, if the weight of evidence is then on the side of weed control, answer them cooperatively and courteously and go ahead.

Dr. E. P. Sylwester has done this beautifully in his roadside spraying campaign in Iowa. He started this in cooperation with the Highway Department in 1948. The unsightly unremoved dead brush along the road started critics talking so vigorously that the campaign was "soft-pedaled" in 1949, resulting in poorer results and greater expense for the Highway Department.

So, this spring, Dr. Sylwester arranged for a printed debate between himself and one of his active opponents in the Sunday Des Moines Register and Tribune, which has a Sunday circulation of nearly half a million. He also defended the roadside spraying program at some 50 meetings throughout the state. As a result, the Iowa roadside spraying program is going ahead full steam, with relatively little criticism. His handling of the program was a model of public relations for all of us.

So far as anything I might say here defending roadside spraying is concerned, I could only say what Dr. Sylwester said, or say it less well. That article has been reprinted several times, most recently in "Down to Earth" (Fall, 1950). I suggest that you get it. It is a trenchant defense of the beauty, safety, and economy of grasscovered roadsides, produced by spraying, as contrasted with the miles of poison ivy, wild carrot, brush, hemp, ragweed, etc., etc., which still constitute the average roadside.

Poisoning by herbicides is a more serious matter. Fortunately our problems are small compared to those of the entomologists, but we definitely do have problems. In the first place, any new thing gets blamed for anything that happens in its vicinity. When Dr. Beard of our Department made a week's tour of northwestern Ohio in 1947 putting on a few spraying and dusting demonstractions with 2,4-D, two reports of poisoning followed - both later shown to be erroneous, but illustrating the tendency to blame troubles onto anything new. Last summer I investigated a case of alleged sheep poisoning by a

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"brush killer" used by an electric coop. Investigation showed that there was no reasonable chance that the spraying was responsible, but the spray truck passed along a little before the sheep took sick, so the two things were linked, both by the farmer and by the veterinarian.

Of the new herbicides, TCA is caustic to the skin and the dinitros are definitely poisonous. The DN's are a serious hazard to the person applying them - death has resulted from long exposure to air contaminated by droplets of DN spray - but so far no complaints of livestock poisoning from DN's have come to our attention, and grazing experimentally sprayed areas with them at four times the recommended doses has given no serious results (8). The same is true of TCA (8).

There have been a few reports of persons who are allergic to or affected by 2,4-D. There is no reason to suppose that at least some of these reports are not true. These are important to the few individuals concerned, and worthy of serious study, but we now have experience enough to say that they are highly exceptional. To help put the matter in proper perspective, in my personal experience I know more people who are poisoned by eggs than I have definitely heard of being unfavorably affected by 2,4-D.

Many tests (8, 11, 12) have shown that stock will consume grass sprayed with 2,4-D and 2,4,5-T readily and without injury under ordinary conditions. These materials have been directly fed to milkcows without injurious effects on the cattle. Most reports also state that there was no effect on the milk - Dr. Grigsby in a letter reporting unpublished work this summer (1950) says that cows fed large quantities of 2,4-D gave milk with a characteristic phenol flavor during the feeding and for 12 hours after the last dose of 2,4-D had been given.

These experiments and the experiences of thousands of farmers with sprayed pastures pretty well dispose of direct 2,4-D toxicity to stock. However, almost all of us have seen sprayed plants eaten that had not been eaten before - all the way from S. M. Raleigh's case of rodents choosing to eat rows of corn treated pre-emergence with 2,4-D to stock eating Canada thistles (C. E. Lee), velvet leaf (J. L. Hutchison), jimson weeds (F. W. Slife), wild parsnip (Geo. Briggs, C. J. Willard), sunflowers (T. F. Yost), docks (Crafts and Harvey), round leaved mallow (N. E. Shafer) and unpalatable weeds in general (Chas. J. Gilbert, H. E. Wood).

Clearly, if any of these weeds were injurous or poisonous, their sudden consumption after spraying could cause sickness or death of the stock. Actual instances are few but suggestive. C. J. Gilbert mentions cases of poisonous range weeds causing death when eaten after spraying. Both Warren Shaw and Oliver Lee report poisoning of cattle from 2,4-D-sprayed wild cherry and if this

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definitely poisonous plant is present in an area to be sprayed, it would certainly be well to keep stock out of it. J. R. Fleetwood reports hearing of a case of swine eating sprayed mature cocklebur with severe sickness following. Crafts and Harvey (4) make a similar report concerning sprayed thistles eaten by lambs. Virgil Freed mentions several instances of ragwort poisoning livestock after being sprayed with 2,4-D. He writes: "Normally, livestock become poisoned on this plant only when they are forced to graze it by lack of other vegetation, particularly in the late winter and early spring months. However, in the instances mentioned above, it was a case of the animal actually seeking out this plant two or three days after spraying and consuming sufficient quantities to be toxic. Plants that had been treated for a period longer than one to three days did not appear to be palatable to the animal and the initial palatability was attributed to the marked increase in sugar that accompanies application of 2,4-D to these plants. Normally the higher sugar content extends from 24 to 72 hours after spraying, thereafter declining steadily." A wise precaution, therefore, would be to keep stock out of sprayed pastures containing any poisonous weeds for 3 to 4 days to a week.

Much the most serious possible 2,4-D poisoning effect that has been reported was due to the accumulation of nitrates in sugar beet leaves that have been sprayed with sub-lethal amounts of 2,4-D. For these data I am almost entirely indebted to an unpublished paper submitted to "Science" by L. M. Stahler and E. Whitehead, which I am abstracting in part.

Nitrate poisoning of stock has been known for some time (1, 2, 5, 7) especially in connection with oat hay. Beet leaves from a Manitoba field damaged by airplane spraying of an adjacent wheat field in 1948 caused the death of several steers. A local veterinarian recognized the symptoms as those of nitrate poisoning. The standard treatment, injection with methylene blue, revived living but recumbent steers within an hour. The leaves were analyzed for nitrate and showed nitrate levels for above those known to be toxic.

In August, 1949, 335 acres of beets on seven farms in North Dakota were sprayed with insecticide contaminated with 2,4-D (15). Through the cooperation of Dr. Helgeson and associates, Dr. Stahler obtained samples of beet leaves from each field and also from three adjacent untreated fields. The leaves from untreated fields averaged 0.22 percent KNO_3; the average of the seven treated fields was 4.5 percent KNO_3, with a range of 1.61 to 8.77 percent. Over 1.5 percent of KNO_3 in the dry matter of forage is considered toxic.

The toxicity of nitrates is not direct, but in the rumen of ruminants, nitrates are reduced to nitrites, which so alter the blood that it cannot carry oxygen from the lungs to the tissues. The blood becomes brown instead of red, giving a gray-blue appearance to the mucous membranes of the nose and mouth. Unless treated

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promptly, death may come in a few hours or less.

Pigweed (Amaranthus retroflexus) and lambsquarters (Chenopodi album) are common weeds closely related to beets which Olson and Whitehead (13) at South Dakota have shown to accumulate nitrates in hazardous amounts, even without 2,4-D treatments. J. W. Zahnley writes me of unpublished work by Harold Jones of Kansas State College, showing that lambsquarters, pigweed, and smartweed (Polygonum sp.) treated with 2,4-D were extremely high in nitrate, while those not treated contained very little. This seemed to be due to the 2,4-D checking the assimilation of nitrates into protein, so that nitrate accumulated in the treated plants, but was used up in the untreated ones. It would surely be risky to spray a pasture containing these weeds. Presumably, if they are killed outright, they will not accumulate more nitrate before death, but with the practical irregularities in spraying we cannot be sure that this will occur. Corn, sorghum, proso, sunflower (Helianthus spp.) gumweed (Grindelia spp.) and spiderwort (Tradescantia spp.) have also been indicated (13) as containing lethal quantities of nitrate under some conditions.

Mr. C. D. Floyd, State Apiarist for Minnesota, reports one rather clear case of poisoning of bees pasturing on mustard sprayed after it came in bloom. He was not able to duplicate the result the next year, even by feeding the bees syrup contaminated by supposedly the same 2,4-D formulation used the year before. However, something killed the bees the preceding year (1948). Mr. Floyd said no reports of injury were received in 1950. Apparently the trouble is at least unusual.

There will be many of these problems, real and imaginary, as weed control continues to develop. Our attitude, as good weed control men, must be to consider every complaint, even those which look absurd, cautiously and carefully, neither saying "Impossible" without investigation, or fearfully accepting unproven responsibility.

There have been many reports of 2,4-D increasing the protein content of grain (6, 9, 14). In general, these effects seem physiologically to be like the increased protein obtained in a dry season: that is, the protein elements in the grain are laid down first, and if the grain does not "fill" well, it will be higher in protein than one that does fill. Usually, at least, the protein per acre has not been increased by spraying.

However, many of the effects on the vegetative parts of plants (10, 3) are not so simple and a wide field is open for the study of the effects of 2,4-D and related compounds on forage. The vegetable men know well the greater development of fiber in asparagus, etc., following 2,4-D treatments. In field crops we have no data as yet, but the problem is highly important.

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ES-274

March 16, 1979

Environmental Sciences Research

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TOXICITY OF ELEVEN HERBICIDES TO DAPHNIDS

20 PAGES IN FULL REPORT

CRI NUMBER

W. M. McCarty

W. M. McCarty 4/3/79

H. C. Alexander 4/6/79

This report is: ☐ INTERIM ☒ FINAL and mainly: ☒ NEW ☐ REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS: (Include in this space references to data books, and to earlier related reports, patents and publications.)

Eleven herbicides were evaluated for acute toxicity to the aquatic invertebrate, Daphnia magna Straus. This study was conducted to procure environmental data on these products.

The 48-hour LC50 values and their confidence intervals for the samples evaluated are as follows:

	Sample	48-Hour LC50
K-2372	2,4-Dichlorophenoxy acetic acid	262 (248-276) mg/L
K-6398	Butyl ester 2,4-D	10.6 (8-14) mg/L
K-44181	DOWANOL* PIB Ester of Silvex	1.2 (0.7-1.6) mg/L
K-74618	DOWANOL PIB Ester of 2,4-D	13.2 (9.9-16.5) mg/L
K-44179	DOWANOL PIB Ester of 2,4,5-T	46. (22-65) mg/L
K-20054	Iso-octyl 2,4-D	18.7 (0.00-33.5) mg/L
M-3604	Formula 40	456 (394-528) mg/L
DR-0132-7817	DMA 6 Unseq.	467 (425-506) mg/L
K-11459	MCP amine	146 (36-223) mg/L
DR-0101-0419	Butoxy ethanol ester of dichlorprop	14.7 (11.7-19.2) mg/L
K-7722	DOWANOL EB ester 2,4-D	32.6 (25.6-43.9) mg/L

(99) 95% Confidence Interval

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Vol. XV, No. 4, October 1951

CHEMICALS IN FOODS: A REPORT TO THE ASSOCIATION OF FOOD AND DRUG OFFICIALS ON CURRENT DEVELOPMENTS. PART II. PESTICIDES

ARNOLD J. LEHMAN, M.D.

Chief, Division of Pharmacology, U. S. Food and Drug Administration

DOW 459562

INTRODUCTION

The pesticides will be discussed under the sectional headings of acute toxicity, dermal toxicity, subacute and chronic toxicity, biochemistry, and pathology. In the tabular summaries to follow it will be necessary to mention many of the individual pesticides a number of times. Since their chemical names are too cumbersome for this purpose the common or short-term designation will be given preference. A list of the pesticides to be considered is given below. In this listing the short term is given first with the more customary chemical designation, wherever applicable, presented in the second column. Because of the diversified character of the pesticides from both the chemical and utility standpoint, a classification based upon either one or the other of these aspects did not appear to be practical; hence, in an attempt to segregate the compounds into some useful order an arrangement was devised which is based partially upon chemical similarity and partially upon use. Chemical names will not be repeated in tabulations and the groupings as presented below will be maintained.

	Plant Products and Derivatives
1. Derris	Rotenone and rotenoids
2. Rotenone	
3. Pyrethrins	Oleoresins
4. Allethrin	Allyl cinerin
5. Nicotine	
6. Sabadilla	Veratrine-like alkaloids
7. Ryania	Alkaloids: ryanodine, ryanine, and others
8. Dihydrorotenone	
	Thiocyanates
9. Thanite	Isobornyl-thiocyano acetate
10. Lethane-60	Beta-thiocyano-ethyl esters of aliphatic acids with 10–18 carbon atoms
11. Lethane-384	Beta-butoxy-beta-thiocyano-diethyl ether
12. Lethane Special	Lethane-60, 3 parts and Lethane-384, 1 part
	Copper
13. Copper chloride	
14. Copper carbonate	
15. Copper sulfate	
	Mercury
16. Calomel	Mercurous chloride
17. Corrosive sublimate	Mercuric chloride

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18. Ethyl mercuric phosphate	
19. Phenyl mercuric triethanol ammonium lactate	
Arsenic	
20. Arsenic trioxide	
21. Potassium arsenite	
22. Paris green	Copper aceto-arsenite
23. Calcium arsenate	
24. Lead arsenate	
Fluorides	
25. Barium fluosilicate	
26. Cryolite	Sodium aluminum fluoride
27. Sodium fluoride	
28. Sodium fluosilicate	
Miscellaneous Metals	
29. Sodium selenite or selenate	
30. Cadmium chloride	
31. Tartar emetic	Potassium antimonyl tartrate
Chlorinated Hydrocarbons	
32. DDT	Dichloro-diphenyl-trichloroethane
33. TDE	Dichloro-diphenyl-dichloroethane
34. DFDT	Difluoro-diphenyl-trichloroethane
35. Methoxychlor	Dimethoxy-diphenyl-trichloroethane
36. TBH	Technical benzene hexachloride
37. ABH	Alpha isomer of benzene hexachloride
38. BBH	Beta isomer of benzene hexachloride
39. DBH	Delta isomer of benzene hexachloride
40. Lindane	Gamma isomer of benzene hexachloride
41. Toxaphene	Chlorinated camphene
42. Chlordane	1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
43. Aldrin	1,2,3,4,10,10 - Hexachloro - 1,4,4a,5,8,8a - hexahydro-1,4,5,8-dimethanonaphthalene
44. Dieldrin	1,2,3,4,10,10 - Hexachloro - 6,7 - epoxy - 1,4,4a,5,6,7,8,8a - octahydro - 1,4,5,8 - dimethanonaphthalene
45. Heptachlor	1,4,5,6,7,8,8 - Heptachloro - 3a,4,7,7a - tetrahydro-4,7-methanoindene
46. Prolan	2-Nitro-1,1-bis(p-chlorophenyl) propane
47. Bulan	2-Nitro-1,1-bis(p-chlorophenyl) butane
Organic Phosphates	
48. Parathion	O,O-Diethyl-p-nitropheryl-thiophosphate
49. Dimethyl parathion	O,O-Dimethyl-p-nitrophenyl-thiophosphate
50. Paraoxone	O,O-Diethyl-p-nitrophenyl-phosphate
51. OMPA	Octamethyl pyrophosphoramide
52. EPN	Ethyl p-nitrophenyl-thionobenzenephosphonate
53. TEPP	Tetraethylpyrophosphate
54. HETP	Hexaethyltetraphosphate
55. ASP-47	Tetraethyldithionopyrophosphate

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56. E-838	Diethoxythiophosphoric acid ester of 7-hydroxy-4-methyl-coumarin
Dinitro Compounds	
57. DNOC	3,5-Dinitro-o-cresol
58. DN-111	2,4-dinitro-6-cyclohexyl phenol dicyclohexylamine
Herbicides	
59. 2,4-D	2,4-Dichlorophenoxy acetic acid
60. 2,4,5-T	2,4,5-Trichlorophenoxy acetic acid
61. Naphthalene acetic acid	
62. Methyl-1-naphthalene acetic acid	
63. Ammate	Ammonium sulfamate
64. Pentachlorophenol	
65. Endothal	3,6-Endoxohexahydrophthalic acid
66. Maleic hydrazide	
Activators	
67. n-Propyl-isome	di - n - Propyl - 6,7 - methylenedioxy - 3 - methyl - 1,2,3,4 - tetrahydronaphthalene - 1,2-dicarboxylate
68. Piperonyl butoxide	(Butylcarbityl) (6-propyl piperonyl) ether
69. Van Dyke 264	n-Octyl-bicycloheptane dicarboximide
70. Sulfoxide	n-Octyl-sulfoxide
71. Piperonyl cyclohexanone	Alkyl-5-(3,4-methylenedioxy)-phenyl-3-3-cyclohexen-5-one
Rodenticides	
72. 1080	Sodium monofluoroacetate
73. Castrix	2-Chloro-4-dimethylamino-6-methylpyrimidine
74. ANTU	Alpha-naphthylthiourea
75. Warfarin	2 - Alpha - phenyl - beta - acetyl - ethyl - 4 hydroxy-coumarin
76. Strychnine	Strychnine nitrate
77. Thallium sulfate	
78. Red squill	
79. Zinc phosphide	
Miscellaneous	
80. Phygon	2,3-Dichloronaphthaquinone
81. Spergon	Tetrachlorobenzoquinone
82. Phenothiazine	Thiodiphenylamine
83. Aramite	Beta' - chloroethyl - beta - (p - tert. - butyl - phenoxy)-alpha-methyl-ethyl sulfite
84. Arasan	Tetramethyl-thiuram-disulfide
85. Fungicide 311 C	Glyoxalidine acetate
86. Dithane	Zinc and sodium salts of ethylene-bis-dithiocarbamic acid

SECTION I. ACUTE ORAL TOXICITY

The acute toxic dose values for the pesticides are listed in Table I. Practically all of the data were obtained in the laboratories of the Division

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of Pharmacology, and, with a very few exceptions, the rat was employed as the test animal. The values for a few of the older compounds such as derris, sodium fluoride, and arsenic trioxide are so well documented in the literature that they were not necessarily checked in this laboratory. In some instances technical difficulties would not permit the calculation of an LD_{50}, hence, in the interest of accuracy the term "approximate LD_{50}" is given as the column heading. Wherever possible the pure compound dissolved in an innocuous solvent was employed, and dosages were administered by stomach tube to fasted animals.

In an attempt to present as much useful information as possible in tabular form, the chief characteristics of the poisoning symptoms, the time elements involved in their appearance and duration, and the clinical course of the poisoning as observed in the animals are briefly summarized. A few additional comments on the various groups of pesticides follow.

Thiocyanates: Except for thanite, the acute toxicity was determined on the commercially available concentrates which are 50% solutions in deodorized kerosene. The LD_{50}'s as tabulated were calculated on the basis of the pure compound.

Copper: Although the compounds listed are not used as pesticides, their toxicity does serve to give some estimation of the toxicity of copper oxychloride, oxide, phosphate, silicate, zeolite, oxychloridesulfate, and Bordeaux Mixture.

Mercury: The general trend of phenyl mercuric compounds is an LD_{50} of 50 mg./kg. or less.

Arsenic: The ratio of toxicity of arsenites to arsenates may be considered as 1:1.6; hence, other arsenical pesticides not listed, such as sodium and zinc arsenites, would have an LD_{50} of about 13–14 mg./kg., and copper, magnesium, and manganese arsenates would be in the 22–23 mg./kg. range.

Miscellaneous Metals: The value for sodium selenite or selenate is calculated on the basis of selenium. The toxic dose of cadmium chloride is listed because this metal is a constituent of at least one fungicide, a combination of cadmium copper zinc chromate and calcium sulfate.

CONTINUED IN THE NEXT ISSUE

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Acute oral toxicity

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Material	Acute oral toxicity LD50	Symptoms of poisoning: Chief Characteristics	Onset	Duration	Clinical Course with Severe Poisoning
Natural products and derivatives					
1. Derris	1000	Frequent convulsive seizures followed by depression of central nervous system	Within minutes to 5 to 6 hours	1 to 3 days	Death by respiratory failure in 24 hours. Fatalities rare after 3rd day although death may be delayed 10 or 12 days
2. Rotenone	132	Same as for derris			
3. Pyrethrins	200	Central nervous system irritation; hyperexcitability, tremors, and convulsions	Usually within ½ hour	10 hours	Death may be delayed for as long as 5 days. Respiratory paralysis is a major cause of death
4. Allethrin	630	Tremors and convulsions	30 minutes	6 hours	Fatalities appear to be rare 24 hours after poisoning. Death due to respiratory paralysis
5. Nicotine	50-60	Clonic convulsions	Within minutes	5 to 20 minutes	Curare-like paralysis of respiratory muscles and death
6. Sabadilla	4020	Retching, muscular spasms, ataxia, coma	Within 5 minutes	24 hours	Symptoms similar to aconite. Death from respiratory or cardiac paralysis may occur within 3 to 10 minutes after the ingestion of a fatal dose
7. Rynnia	750	Depression of central nervous system	5 to 8 minutes	2 to 10 hours	Continuous depression up to terminal convulsions. Death usually within 24 hours although large doses may be fatal within the hour after ingestion
8. Dihydrorotenone	220	Same as for derris			
Thiocyanates					
9. Thanite	1060	Deep depression, cyanosis, dyspnea and tonic convulsions	Within a few minutes	Rapid collapse within minutes	Occasionally symptoms may be delayed for a few hours. Death is due to respiratory paralysis
10. Lethane-60	500				
11. Lethane-384	90				
12. Lethane Special	400				
Copper					
13. Copper chloride	140	Violent retching, muscular spasms and collapse	Within a few minutes	If dose is retained could be fatal in 1 hour	Symptoms of gastrointestinal irritation may subside, but death may follow several days later because of damage to important organs such as liver and kidneys
14. Copper carbonate	160				
15. Copper sulfate	300				
Mercury					
16. Calomel	(210)				Symptoms of mercurialism at this dose but no deaths
17. Corrosive sublimate	37	Shock, dysentery, and anuria	Immediate	Symptoms persist till death	Shock effect of the irritant may be fatal within 24 hours. Acute kidney damage may be fatal by the third day
18. Ethyl mercuric phosphate	30	Similar to corrosive sublimate			
19. Phenyl mercuric triethanol ammonium lactate	20				

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ARSENIC					
20. Arsenic trioxide 21. Potassium arsenite 22. Paris green 23. Calcium arsenate 24. Lead arsenate	13 14 23 20 100	Violent gastroenteritis, diarrhea, rice water stools	Almost immediately	A few hours to several days	Death from exhaustion and dehydration. Convulsions and general paralysis may occur before onset of gastroenteritis. Death within a few hours to several days
Fluorides					
25. Barium fluosilicate 26. Cryolite 27. Sodium fluoride 28. Sodium fluosilicate	175 200 200 125	Abdominal distress, diarrhea, cyanosis, dyspnea, fibrillation of skeletal muscles	Immediately and up to 6 hours	Stupor and weakness may persist for 23 hours	In fatal poisonings death can result in a few minutes
Miscellaneous Metals					
29. Sodium selenite or selenate	2.5	Garlic breath, nervousness, central nervous system depression	Within 15 minutes	12 to 18 hours	Death due to respiratory failure
30. Cadmium chloride	83	Salivation, vomiting, diarrhea	15 to 30 minutes		Gastroenteritis may be a contributing cause of death.
31. Tartar emetic	115	Parallels those given for arsenic poisoning			Death within a few hours to several days
Chlorinated hydrocarbons					
32. DDT	250	Extreme excitability, tremors, twitching, convulsions, coma, death	Within 30 minutes	24 hours	Time interval for a fatal outcome is variable, but death usually occurs within 24 hours
33. TDE	3400	Lethargy	Within 24 hours	3 to 4 days	Convulsions do not appear to be a major symptom. If death has not occurred by the 4th day prognosis appears to be favorable
34. DFDT	1120	Similar to DDT			Apparently the fluorine part of the molecule does not contribute to the symptoms of acute poisoning
35. Methoxychlor	6000	Largely depression of central nervous system	Within 24 hours	2 to 4 days	Tremors have been noted but are not a prominent symptom
36. TBH	600	Convulsions which may give way to a central nervous system depression	Within 30 minutes	2 weeks	Symptoms may persist for 2 weeks and deaths have been observed late in the 2 weeks observation
37. ABH	500	Hyperexcitability and convulsions	About 1 hour	2 weeks	Deaths delayed up to 2 weeks with convulsions persisting to the end
38. BBH	6000	Principally tremors	2 to 6 hours	2 days	Death occurs between the 2nd and 8th day
39. DFH	1600	Depressant to the central nervous system	1 to 2 hours	2 days	Death may be delayed to between the 2nd and 6th day

Chlorinated hydrocarbons—Continued					
40. Lindane	125	Hyperexcitivity and convulsions	20 minutes	2 hours	Death usually within a two-hour per after ingestion. Convulsions pers to the end
41. Toxaphene	60	Hyperexcitivity, tremors, and convulsions	1½ hours	24 hours	Most of the fatalities occur within hours. Delayed deaths have been no up to the 6th day
42. Chlordane, technical	457	Hyperexcitivity, tremors, and convulsions	15 minutes	2 days	Tremors begin in 15 minutes; convulsio appear after about 24 hours and m persist for 2 days; deaths may be d layed up to the 6th day
43. Aldrin	67	Tremors and convulsions	Within 1 hour	2 days	Time of death variable; may be delay up to the 6th day
44. Dieldrin	87	Tremors, muscular spasms, convulsions	Usually within the hour	2 days	Deaths may be delayed up to the 6th d or longer
45. Heptachlor	60	Tremors and convulsions	30 minutes to an hour	2 days	Similar to aldrin, dieldrin and chlorda
46. Prolan	4000	Tremors and convulsions	Within about 1 hour	Prolonged for days	Persistent convulsions up to time death which may not occur within hours
47. Bulan	330	Convulsions	About 2 hours	4 hours	Fatalities usually occur within 4 ho

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Organic Phosphates					
48. Parathion 49. Dimethyl parathion 50. Paraoxone 51. OMPA 52. EPN 53. TEPP 54. HETP 55. ASP-47 56. E-605	3 15.2 7.8 13.5 14.5 1.3 7 5 19	Generalized fibrillary tremors, salivation, lacrimation, diarrhea and convulsions	15 minutes to 1 hour	12 hours	The symptomatology of the individu organic phosphates is similar. Death usually occur in 1 to 24 hours
Dinitro Compounds					
57. DNOC 58. DN-111	25 220	Increased respiratory rate	Within the hour	24 hours	Time of death variable but usuall within 24 hours with hyperpyrexi contributing cause
Herbicides					
59. 2,4-D	500	Ataxia, myotonia, gastrointestinal irritation	30 minutes	Several hours	Death occurs several hours after inges tion
60. 2,4,5-T	300	Similar to 2,4-D			
61. Naphthalene acetic acid 62. Methyl-1-naphthalene acetic acid	1000 2140	Gastroenteritis, depression and paralysis	30 minutes	Less than 24 hours	Most fatalities occur within the first 4 hours
63. Ammate	3900	Listlessness	Within minutes	24 hours	Tremors have been noted with very high doses and death within 10 minutes. Survival after 24 hours is favorable for complete recovery
64. Pentachlorophenol	73	Tremors and convulsions	30 minutes		

65. Endothal	65.5	Muscular weakness	30 minutes	24 hours	Deaths occurred between 4 and 24 hour
66. Maleic hydrazide	4000	Tremors, muscular spasms	10 to 60 minutes	3 hours	Deaths within 3 hours
Activators					
67. n-Propyl isome	15000	Depression			
68. Piperonyl butoxide	11000	Depression	20 minutes	Several days	Delayed deaths up to one week
69. Van Dyke 264	2000	Some hyperexcitability followed by depression	20 minutes	2 days	Most fatalities occur within the 2-day period
70. Sulfoxide	2000	Tremors, severe depression, coma	10 to 20 minutes	24 hours or more	Continuous severe depression till death Survivals may have severe symptoms extending beyond the 21-hour period
71. Piperonyl cyclohexanone	6200				Death usually within three days
Rodenticides					
72. 1080	1.7	Tremors, convulsions	45 minutes	1 week	Intermittent convulsions for as long a 10 days before death
73. Castrix	1.7	Convulsions	45 minutes	12 hours	Survival for more than 12 hours appear to be a favorable prognostic sign
74. ANTU	6	Dyspnea; occasionally convulsions	15 minutes	24 hours	Death usually occurs within 24 hours

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75. Warfarin	100	Severe depression; generalized hemorrhages			Deaths are usually delayed but in severe poisoning death could occur within half hour after ingestion
76. Strychnine	10.2	Tonic convulsions	10 minutes	12 hours	Deaths from medullary paralysis and exhaustion and usually occur within 12-hour period
77. Thallium sulfate	25	Depression	48 hours	72 hours	Most of the fatalities occur within th 72-hour period. Renal damage is th principal cause
78. Red squill	300	Central nervous system stimulation	12 hours	24 hours	Animals exhibit circular movements; re peated rotation on long axis of body Majority of deaths fall within the 24 hour period
79. Zinc phosphide	450	Depression	24 hours	72 hours	Most deaths occur within 72 hours
Miscellaneous					
80. Phygon	1000	Diarrhea and severe depression			Deaths occur within 24 hours
81. Spergon	4200	Depression			Depression may be prolonged for day before death
82. Phenothiazine	5000	Secondary anemia			Recovery from temporary secondary anemia if poisoning is not serious; n residual toxic effects
83. Aramite	6300	Depression	3 hours	3 days	Delayed deaths over 1 week
84. Arasan	865	Hyperexcitability, severe diarrhea			Most deaths occur within 5 days
85. Fungicide 341 C	3720	Depression, hemorrhage from the nose			Delayed deaths up to 2 weeks
86. Dithane	5000	Diarrhea			

ACUTE AND SUB-ACUTE ORAL TOXICITY OF

2,4-DICHLOROPHENOXYACETIC ACID FOR DOGS

DOW 2239611

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2,4-dichlorophenoxyacetic acid (2,4-D) is known to act as a plant hormone and as a herbicide (1, 2, 3). In one initial report 2,4-D was mentioned to be non-toxic to animals and humans when administered orally (1). However, large doses produced symptoms in mice, rats, rabbits and dogs, which simulated those seen in clinical uremia. More detailed observations were reported by Hill and Carlisle (5) who studied the toxicity of 2,4-D acutely and sub-acutely in various species of animals.

There are no reports in the literature on the acute oral toxicity or chronic oral toxicity of 2,4-D in dogs. The present study was designed, therefore, to observe the acute effects of single doses of 2,4-D and the chronic effects of 2,4-D when administered orally for 90 days. Observations were made on the mortality, symptomatology, body weight, blood count and gross pathology of the animals. A microscopic study of the tissues is under

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may but at the present time is not completed.

Methods

Adult mongrel dogs of both sexes were used. Most of the animals were housed in the laboratory for a period of 2 to 3 months before the study was started. During this control period they were immunized against distemper with the Green vaccine. The dogs were fed a standard stock diet (Friskies) ad libitum. The 2,4-D used was commercial material with a purity of 98.5%.

In the acute studies the calculated dose of 2,4-D was administered as a single oral dose in capsules. The dogs were observed for a period of 14 days at which time survivors were autopsied. In the chronic study the 2,4-D was administered orally in capsules, 5 days a week over a 13 week

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period. Each capsule was imbedded in a 4 to 5 gram piece of commercial canned dog food. The animals consumed this readily, thus avoiding the continued trauma of the stomach tube. During the study the animals were weighed twice a week. Control blood counts were taken before the administration of the drug, on the 30th day and on the 60th day at the completion of the study. Hemoglobin was determined with a photoelectric colorimeter (6). Upon the death or at the completion of the study, the animals were autopsied, tissues were taken from the lung, heart, liver, kidney, adrenal, spleen, thyroid, small intestine ovary or testes and fixed in 10% formalin.

RESULTS

1. ACUTE ORAL TOXICITY

A. Symptoms produced. The effect of various doses of 2,4-D on survival for 14 days is shown in Table 1. The oral LD_{50} is approximately 100 mg/kgm.

In dogs that died the symptoms produced by 2,4-D varied from a mild ataxia and stiffness in the hind legs to a definite myotonia. Initial effects were often noted 6 hours after the oral administration of 2,4-D. The animals were more quiet than normal and a slight ataxia was occasionally present at this time. The hind legs were always affected first, the forelegs later or not at all. There was usually a progressive increase in spasm in the hind legs associated with increasing ataxia and some dogs spontaneously extended the hind limbs in a spastic movement lasting a few seconds. The knee jerk was either normal or hyperactive.

In the early stages of acute poisoning the animals were usually quiet. If the animal is lifted and made to walk the spasm and ataxia are noticed, but when walked for a while the spasm and ataxia tend to decrease. In the later stages of intoxication the animal, when placed on his feet, in

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unable to stand and sits or lies in an outward position with spastic limbs, particularly spasm of the hind limbs. Other dogs will stumble and roll on the floor in attempts to right themselves. An occasional dog has shown evidence of irritation or pain when the skin over the back of the neck is grasped. Some dogs, when held up by the shoulders would show extension and crossing of the hind limbs. As the toxic effects of the drug progressed the dogs eventually refused the various types of food offered. Although occasional diarrhea was obtained, vomiting was not produced.

Such symptoms may also be seen in dogs that survive the lower acute doses of 2,4-D for 14 days (Table I). The symptoms in such cases are relatively mild and were limited to spasm in the hindlegs and a slight ataxia.

b. Body weight. The animals dying generally showed a loss of weight, the loss being least in animals that died during the first 4 days of intoxication (Table I). There was no loss of weight in the animals that received 25 mg. of 2,4-D per kilo. Although food intake was not measured, the dogs ate less food than normal and those that died refused even canned

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...ed towards the end of their survival.

2. Gross pathology. The chief gross changes noted were in the small intestine. The intestinal mucosa in some cases showed a mild to diffuse ...dness (Dog #, 71, 75, 41). Dog 75 had a single indurated ulcer in the ...cecum. Dog 41 showed what appeared to be occasional small circumscribed ...emorrhagic areas in the lungs. In dog 76, 90% of the middle left lobe was a mottled, purple-black. Similar changes, but less extensive, were present in the other lobes. Dog No. 75 showed similar gross changes in the middle right lobe and left lobes.

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II. CHRONIC ORAL TOXICITY

1. Mortality. All dogs receiving 2, 5 or 10 mgms. of 2,4-D per kilo survived the 90 day test period. Both dogs receiving the 20 mgm. per kilo died during the study. The same total daily dose divided and administered twice a day to two dogs caused death, after a longer time interval, in only one animal (Table 2).

2. General symptoms. Dogs that survived the oral administration of 2,4-D for 90 days were free of any symptoms.

The three dogs that died while receiving the highest dose of 2,4-D showed symptoms and signs differing somewhat from those observed in the acute studies. The dogs were quiet, weaker and less responsive than normal. Muscle tonus was higher in the hind legs, particularly on passive extension. The hind legs were held more stiffly than usual when walking and a slight ataxia was present. During the last 2 to 3 days of their survival 2 of the dogs showed difficulty in chewing or swallowing and eventually even refused a small bolus of canned dog food (usually readily consumed). There was also some oozing of blood from the gums and buccal mucosa.

3. Body weight. Changes in body weight are listed in Table 2. The only significant change was observed in the dogs that failed to survive the study. The weight loss in these animals began 7 to 12 days before the death of the dog.

4. Blood count. The administration of 2,4-D did not have any effect on the hemoglobin or red cell count of animals that survived or died during the study (Table 3). Of the animals that died, one dog (No. 104) showed a moderate decrease in total white cell count. However, as a similar change was observed in a control dog (No. 62) it was concluded that this was not a significant effect of the drug. The differential count was not

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significantly affected by 2,4-D with the possible exception of dog 64. This dog showed a marked drop in the per cent of lymphocytes before death. However, a similar change was not present in dog No. 104 just before death.

5. Gross pathology. Gross changes in the internal organs were minor. In dog 61 the renal capsule stripped with difficulty, and a few isolated red areas (1-2 mm.) were present in the duodenum. Dog 91 showed a slight diffuse redness of the duodenal mucosa.

6. Organ weights. There was no significant change in the weight of the thyroid, adrenal, heart, liver or kidney in animals that survived the 90 day test period. An increase in some of the organ weights can be noted in dogs 64, 91 and 104 that received the highest dose of 2,4-D and who died during the experiment.

Discussion. The symptoms produced by the high single acute doses of 2,4-D are consistent with those observed by Bucher (4). The general symptoms seem to be quite similar in either the mouse, rat, rabbit or dog, and are characteristic of a clinical myotonia. Other effects occasionally observed in the dog are sneezing, and rubbing the eyes, or occasionally diarrhea. Vomiting was not observed in the present study. Some of the dogs dying of the acute doses showed a mild to diffuse redness of the duodenum mucosa and occasionally areas in the lung that appeared to be hemorrhagic. The higher doses of the material produced anorexia and consequent weight loss. Death from large single oral doses of 2,4-D is delayed and occurs days after the initial administration. The irritation and pain observed in some dogs when the skin of the back of the neck is grasped may indicate neuromuscular irritation, and the extension and crossing of the hind legs in some dogs, when lifted by the shoulders, may point to a spinal or central lesion.

In the chronic study the dogs survived doses of 2, 5 and 10 mg. of 2,4-D per kilo of body weight. Doses of 20 mg. per kilo, however, did

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produce death when administered over a period of 15 to 84 days. This is in conformity with sub-acute studies in dogs performed by Hill and Carlisle (5). They administered 2,4-D intravenously in doses of 25 or 50 mg. per kilo for 6 injections. Such animals died or were sacrificed between the 3rd and 15th day of the study. It is evident then that there is some cumulative action of 2,4-D in the studies of Hill and Carlisle and in the present study with the 20 mg. per kilo dose.

The dogs surviving the chronic doses of 2,4-D did not show any significant change in hemoglobin or blood count. The doses of 2,4-D did not significantly affect the white blood count. In one animal that died there was a marked fall in the per cent of lymphocytes in the differential count before death. Hill and Carlisle (5), administering 25 or 50 mg. of 2,4-D per kilo intravenously for six days, have observed a fall in the polymorphonuclear leucocyte count and in the per cent of lymphocytes, although not in all dogs.

The symptoms produced in animals dying from the 20 mg. per kilo dose of 2,4-D differed somewhat from those seen in the acute studies. With the chronic administration the animals exhibited chiefly a stiffness of the hind legs and ataxia, weakness, and bleeding from the gums. Some of the animals also showed difficulty in chewing or swallowing.

Summary. 1. The acute oral LD_{50} of 2,4-D is approximately 100 mg. per kilo. Doses in this range or higher produce a definite myotonia which was accompanied by anorexia and a loss of weight.

2. In chronic oral feeding the animals survived doses of 2.5 and 10 mg. per kilo for 90 days. Such doses were without effect on body weight, blood count or organ weight. Occasionally upon autopsy some redness of the duodenum mucosa was noted. Three of the 4 dogs receiving 20 mg. per kilo of 2,4-D died during this experiment. Evidence of clinical myotonia

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was less in these dogs with such animals also showed some bleeding from the gums and necrotic changes in the buccal mucosa accompanied by difficulty in chewing and swallowing. In the animals that died there was no significant change in the blood picture except for a decrease of lymphocytes in one dog.

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Table I

Effect of single oral doses of 2,4-D in dogs

Dog No.	Dose mg/K	*Died day	Mortality	Wt loss %	Symptoms*
[illegible]	400	2	2/2	-0.1	+
[illegible]	400	3		-0.8	++
[illegible]	350	8	3/3	-1.8	+++
[illegible]	350	7		-2.0	++
[illegible]	350	4		-0.4	++
[illegible]	100	5	2/4	-2.5	+
[illegible]	100	9		-2.0	0
[illegible]	100	9		-1.7	+
[illegible]	100	5		-2.4	0
[illegible]	200	5	0/3	0.0	0
[illegible]	200	5		+0.2	0
[illegible]	200	5		+0.2	0

* 5 = survival for 14 day test period.

* column indicates general severity of symptoms as discussed in text. 0 = no change from normal.

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Table 2

Body weight and survival of dogs fed 2,4 D for 90 days

Dog No.	Dose mg/K	Weight Initial Kgm	Weight Final Kgm	Weight Diff. Kgm	Day Died
65F	none	14.9	16.4	+2.7	S
62F	none	9.3	10.9	+1.6	S
51M	2	6.8	9.5	+2.7	S
61F	2	15.2	13.8	-1.4	S
52M	5	9.5	9.5	0.0	S
58F	5	12.0	12.5	+0.5	S
54M	10	14.7	14.8	+0.1	S
63M	10	11.6	11.9	+0.3	S
92M	10	17.2	17.8	+0.6	S
56M	*20	13.0	13.7	+0.7	S
64F	*20	9.8	5.9	-3.9	49
91M	20	12.2	10.1	-2.1	18
104M	20	13.4	9.7	-3.7	25

* = daily dose divided [illegible] in A.M. and ½ in P.M.

S = survived 90 day [illegible]

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Table 3

Effect of 2,4 D on hemoglobin, total blood and differential counts

Dog No.	Dose mg/K	Day	Hb. gms%	RBC mills	WBC	Differential : cnt Neut %	Lymph %	Mon %	Eos %	Baso %	Juvenile %
657	none	0	13.20	5.72	17,800	56	34	3	3	4	
		30	13.82	5.93	15,600	64	29	3	3	1	
		90	13.70	6.29	13,950	71	27	2	0	0	
627	none	0	12.80	6.01	13,900	86	11	3	0	0	
		30	14.72	6.66	13,250	79	12	7	0	2	
		90	14.85	6.16	7,150	79	21	0	0	0	
5Am	2	0	5.12	3.47	13,550	83	12	0	0	0	
		30	6.76	3.88	18,500	57	12	0	0	1	
		90	12.16	5.95	15,400	95	14	0	0	0	
617	2	0	13.31	5.84	16,650	58	33	0	0	9	
		30	14.21	5.98	17,850	58	25	8	0	9	
		90	14.74	6.07	15,550	79	20	0	0	0	
5Am	5	0	10.43	6.55	20,450	71	20	2	0	7	
		30	12.67	6.47	16,200	67	23	3	0	7	
		90	10.28	5.82	15,150	86	12	2	0	0	
587	5	0	14.08	5.92	14,250	69	22	4	0	5	
		30	14.53	5.76	15,550	62	25	3	0	10	
		90	13.57	6.38	16,400	90	10	0	0	0	
5Am	10	0	14.40	6.15	17,950	79	15	6	0	0	
		30	13.95	6.24	17,300	63	29	8	0	5	
		90	14.36	7.23	11,650	73	21	6	0	0	
657	10	0	14.46	6.39	15,200	56	41	3	0	0	
		30	14.78	6.21	19,500	65	28	4	0	3	
		90	14.19	7.28	11,200	93	3	2	0	0	
5Am	18	0	11.[illegible]	[illegible].61	15,000	38	37	3	0	22	
		30	11.96	[illegible].12	17,500	22	54	2	0	20	
		90	12.93	[illegible].43	13,600	35	33	7	0	[illegible]	
5Am	20	0	13.43	5.32	13,300	68	27	5	0	4	
		30	12.44	6.16	15,400	66	28	4	0	2	
		90	11.[illegible]	6.35	12,100	79	29	0	0	0	
617	20	0	13.18	5.26	14,850	58	41	1	0	1	
		30	14.46	5.88	19,700	47	46	4	0	3	
		49	14.39	5.47	18,600	79	18	2	0	0	19
5Am	20	0	13.20	5.72	15,200	59	34	4	0	3	11
14Am	20	0	14.98	6.47	13,150	78	11	1	0	12	
		25	14.59	6.34	9,100	69	27	4	0	1	8

• = daily dose divided and administered ½ in A.M. and ½ in P.M.

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Table 4

Organ weights of dogs fed 2,4 D for 90 days
Based on final body weight

Dog No.	Mg/K	Kidney wt g	Kidney g/Kg	Liver wt g	Liver g/Kg	Heart wt g	Heart g/Kg	Adrenal wt g	Adrenal g/Kg	Thyroid wt g	Thyroid g/Kg
659	none	85	5.18	468	28.5	107	6.53	1.5	0.092	1.0	0.061
639	none	49	4.50	375	34.4	76	6.98	1.4	0.128	0.6	0.055
5IM	2	49	5.14	240	25.3	55	5.79	0.9	0.095	1.5	0.158
615	2	54	3.91	310	22.5	83	6.12	1.4	0.101	1.6	0.116
5IM	5	23	2.42	223	23.4	56	5.90	0.8	0.084	0.6	0.063
589	5	48	4.00	331	26.5	81	6.48	1.6	0.128	0.9	0.072
5IM	10	56	3.78	321	21.7	100	6.76	1.5	0.101	0.9	0.061
638	10	38	3.19	305	25.6	83	6.98	1.1	0.092	0.5	0.042
9IM	10	89	5.00	613	34.4	120	6.74	1.4	0.079	---	---
5IM	*20	71	5.18	289	21.1	97	7.08	1.1	0.080	0.8	0.058
645	*20	66	7.80	218	35.6	55	9.32	1.2	0.203	---	---
9IM	20	70	6.93	302	29.9	76	7.52	1.1	0.109	---	---
10IM	20	74	7.63	500	51.6	89	9.17	2.1	0.217	---	---

* = daily dose divided and administered ½ in A.M., and ½ in P.M.

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CHEMICALS IN FOODS: A REPORT TO THE ASSOCIATION OF FOOD AND DRUG OFFICIALS ON CURRENT DEVELOPMENTS. PART II. PESTICIDES SECTION II. DERMAL TOXICITY

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Pesticides may have uses other than those related to agriculture. A number of them may find clinical application in the control of ectoparasites on man and animals, some may find use as insect repellents, and still others may be useful in treating fungus diseases in human and veterinary medicine. The development of these various uses involves application to the skin; consequently, dermal toxicity studies are of considerable importance. Many of the pesticides are not amenable to clinical use, hence, the list of compounds investigated for dermal toxicity is not as extensive as that presented in Section I dealing with acute toxicity.

The general method of approach in evaluating dermal toxicity was to determine the effect of a single acute dermal exposure, the pesticide being employed in the dry form or in solution in an innocuous solvent, dimethyl phthalate. If these initial tests indicated a favorable toxicity, subacute studies were undertaken. These were either daily applications of a series of dosage levels for a period of 21 or 90 days, or occasionally wear-test experiments in which a definite quantity of the compound under test was incorporated in each square foot of cloth and the animal permitted to wear the treated cloth as a jacket for 21 days. Only limited wear-test data are included in the tabular summaries. Frequently animals failed to survive the scheduled 21- or 90-day test period. This is shown in Table III by indicating the number of doses which were fatal to the animals.

Dosage levels were determined to a great extent by the proposed use of the compound. If the results of single or multiple exposure experiments indicated that the compound was too toxic to warrant further consideration, additional experiments were not done. This accounts for the numerous instances of listing dermal toxicity values as less than (<) the value presented in the tables.

Identity of the compounds listed in Tables II and III will be found in the preceding article of this series.[1]

In summarizing the results it may be stated that insecticidal materials which are solids or formulated as wettable powders and dusts are poorly absorbed through the skin and a single massive exposure may not be serious. At least, if prompt remedial procedures are invoked immediately after large quantities are spilled on the skin the chances of injury appear

[1] Quarterly Bull., Assn. of F. and D. Officials of U. S., XV, No. 4, p. 122.

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TABLE II

Dermal toxicity: Single acute (24 hour) exposure in rabbits

PESTICIDE	FORMULATION	APPROXIMATE LD_{50} MG./KG.	DERMAL EFFECTS	SYSTEMIC EFFECTS
Plant products and derivatives				
Rotenone	10% technical grade in dimethyl phthalate	>940	Severe skin irritation	Slight body weight loss, generalised weakness, partial paralysis and incoördination of extremities
Pyrethrins	20% pyrethrins in soya bean oil	>1880	No significant local skin effects; severe irritation of abraded skin	No systemic effects
Allethrin	See table III			
Nicotine	Undiluted alkaloid	50	No local skin effects	Convulsions, respiratory failure and death
Ryania	In powdered form	>4000	No effect	No systemic effects
Thiocyanates				
Thanite	Undiluted technical grade	6.0 ml.	No local skin effects	Little evidence of poisoning until lethal dose is reached
Lethane-60	50% concentrate in petroleum distillate	10.0 ml.	Moderate skin irritation due to petroleum distillate	Little evidence of poisoning until lethal dose is approached
Lethane-384	50% concentrate in petroleum distillate	0.25–0.5 ml.	No significant local irritation	Extremely toxic; no warning signs of poisoning until lethal dose is approached. For symptoms see Table I
Lethane Special	Lethane-60, 3 parts Lethane-384, 1 part	1.0 ml.	Slightly irritating due to petroleum distillate	As for Lethane-60 and Lethane-384
Chlorinated hydrocarbons				
DDT	Microtised powder 50% wettable powder Dry technical grade		No effect	Apparently not absorbed, hence no evidence of toxicity
DDT	30% technical grade in dimethyl phthalate	>2820	No local skin irritation	Slight tremors, anorexia, and emaciation. Complete recovery in 5 to 6 days
TDE	20% technical grade in dimethyl phthalate	1200	Moderate skin irritation	Hyperexcitability and convulsions prior to death. Fatal outcome delayed 7 days post exposure
TDE	Dry technical grade	>4000	Moderate skin irritation	Symptoms of poisoning but no deaths; recovery in 5 to 6 days
Methoxychlor	30% technical grade in dimethyl phthalate	>2820	Slight skin irritation	No symptoms of toxicity
TBH	20% in dimethyl phthalate	>1880	Moderate skin irritation especially of abraded areas	Hyperexcitability indicating some absorption

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TABLE II—*Continued*

PESTICIDE	FORMULATION	APPROXIMATE LD_{50} MG./KG.	DERMAL EFFECTS	SYSTEMIC EFFECTS
Chlorinated hydrocarbons—*Continued*				
Lindane	Dry form	>4000	Moderate skin irritation	Severe symptoms, convulsions, recovery in 5 to 8 days
	2% in dimethyl phthalate	>188	No significant local irritation	Hyperexcitability progressing into convulsions and death
	1% in vanishing cream base	50	No significant local irritation	
Toxaphene	Dry (waxy) form	>4000	Moderate skin irritation	Hyperexcitability, but no deaths; recovery in 5 to 6 days
	20% in dimethyl phthalate	<780	Moderate skin irritation	Tremors leading to convulsions and death
Chlordane	20% in dimethyl phthalate	<780	Severe skin irritation	Tremors, convulsions and death
Aldrin	4% in dimethyl phthalate	<150	No skin irritation	Severe tetanic type of convulsions and death
Dieldrin	4% in dimethyl phthalate	<150	No skin irritation	Like aldrin
Heptachlor	Dry powder	2000	No skin irritation	Severe anorexia, hyperexcitability, convulsions and death
	20% in dimethyl phthalate	<780	No skin irritation	As for the dry powder
Prolan	Undiluted technical grade	>4000	No evidence of skin irritation	No evidence of gross systemic effects
Bulan	Undiluted technical grade	>4000	As for prolan	As for prolan
Organic phosphates				
Parathion	Undiluted technical grade	40–50	No skin irritation with any of these compounds	Extreme weakness, gastrointestional disturbance prior to death
Dimethyl parathion	Undiluted technical grade	300–400		
Paraoxone	Undiluted technical grade	10		
OMPA	20% in water	<780		
EPN	Undiluted technical grade	30–50		
TEPP	Undiluted technical grade	5		
ASP-47	Undiluted technical grade	5		
E-838	Undiluted technical grade	300		
Herbicides				
2,4-D	15% water solution as ammonium salt	1400	Slight edema of skin	Ataxia and muscular weakness prior to death
	15% of free acid in dimethyl phthalate	>1400	Slight edema of skin	Mild symptoms as above but no deaths
	15% of active ingredient in a commercial formulation	<800	Moderate skin irritation especially of abraded areas	Severe depression, muscular paralysis and death

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TABLE II—*Concluded*

PESTICIDE	FORMULATION	APPROXIMATE LD_{50} MG./KG.	DERMAL EFFECTS	SYSTEMIC EFFECTS
Herbicides—*Continued*				
Endothal	2% in water	100	Severe skin irritation especially of abraded areas	Anorexia and hematuria before death
Maleic hydrazide	Technical grade in powder form	>4000	No local skin effects	No systemic effects
Activators				
n-Propyl isome	4% in dimethyl phthalate	>375	No skin irritation	No systemic effects noted at this dosage
Piperonyl butoxide	20% in dimethyl phthalate	>1880	No skin irritation	Hyperexcitability and convulsions but no deaths
Van Dyke 264	5% in dimethyl phthalate	>470	Slight irritation of skin	Slight anorexia and weight loss
Sulfoxide	Undiluted technical grade	>9.0 ml.	Moderate skin irritation	No systemic effects
Piperonyl cyclohexanone	See table III			

TABLE III

Dermal toxicity: Multiple (repeated daily) exposure in rabbits

PESTICIDE	APPROXIMATE LD_{50}, MG./KG.	REMARKS
Plant products and derivatives		
Rotenone	100–200	No deaths at 100 mg. during a 21-day daily exposure period. No survivors after 12 to 14 doses at 200 mg.
Pyrethrins	200–400	No deaths at 200 mg. during a 21-day daily exposure period. No survivors after 8 to 14 doses at 400 mg.
Allethrin		No injury up to 1 gm. per square foot of cloth during a 21-day wear test
Nicotine	40	Based on a 21-day test. In a 21-day wear test no survivors after one exposure at 2 gm. per square foot of cloth; no deaths at the 1 gm. per square foot level
Dihydrorotenone	100–200	No deaths at 100 mg. during a 21-day daily exposure period. No survivors after 10 doses at 200 mg.

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TABLE III—*Continued*

PESTICIDE	APPROXIMATE LD_{50}, MG./KG.	REMARKS
Thiocyanates		
Thanite	< 1.0 ml.	No survivors after 4 to 5 doses at this level; effects of lower doses not determined
Lethane-60	< 1.0 ml.	No survivors after 5 doses at this level; effects of lower doses not determined
Lethane-384	0.1 ml.	Approximately half of the animals died after 8 doses during a 21-day daily exposure period
Lethane-384 Special	0.5 ml.	
Chlorinated hydrocarbons		
DDT	150	Based on a 90-day exposure period. Intercurrent infections resulting from lowered resistance brought about by the chronic posioning effects of DDT appeared to be contributing cause of death. No survivors at 300 mg. after 10 to 14 doses
TDE	200–400	Severe symptoms but no deaths at 200 mg. during a 90-day exposure period. No survivors after 6 doses at 400 mg.
Methoxychlor	600	Based on a 90-day exposure period. At 1200 mg. all animals died after 7 to 19 doses
TBH	< 200	No survivors after 5 to 8 doses. Effects of lower doses not determined
Lindane	20–50	Severe symptoms but no deaths at 20 mg. during a 21-day exposure period. No survivors after 2 to 9 doses at 50 mg.
Toxaphene	40	Based on a 90-day exposure period
Chlordane	20–40	At 20 mg. one animal died after 51 doses during a 90-day exposure period. No survivors at 40 mg. after 10 to 26 doses
Aldrin	< 5	No survivors after 4 to 10 doses. Effects of lower doses not determined
Dieldrin	< 5	No survivors after 14 doses. Effect of lower doses not determined
Heptachlor	< 20	No survivors after 14 doses. Effect of lower doses not determined
Prolan	400–800	Symptoms but no fatalities at 400 mg. during a 21-day exposure period. No survivors at 800 mg. after 6 doses
Bulan	<200	No survivors after 3 to 8 doses. Effects of lower doses not determined
Parathion	5–15	Severe symptoms at 5 mg. during a 21-day exposure period. No survivors at 15 mg. after 3 to 5 doses

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DOW 8820955

TABLE III—*Continued*

PESTICIDE	APPROXIMATE LD_{50}, MG./KG.	REMARKS
Organic phosphates		
Dimethyl parathion	25–100	Symptoms of poisoning but no deaths at 25 mg. during a 21-day exposure period. No survivors after 2
Paraoxone	< 1	No survivors after 14 to 15 doses. Effects of lower doses not determined
EPN		Death of one animal in the group exposed at the rate of 1 gm. per square foot of cloth towards the end of a 21-day wear test
TEPP	< 5	No survivors after 9 to 13 doses. Effects of lower doses not determined
ASP-47	1–2	Severe symptoms but no deaths at 1 mg. during a 21-day exposure period. No survivors after 3 doses at 2 mg.
E-838	10–40	Severe poisoning but no deaths after 40 doses at 10 mg. during a 90-day exposure period. No survivors after 11 to 15 doses at 40 mg.
Herbicides		
2,4-D		No toxicity noted after 1 ml./kg. of a commercial product containing 15% of the active ingredient applied daily for 10 doses
Endothal	>40	No significant systemic effects at this dose during a 21-day exposure period. Higher doses were not tried
Maleic hydrazide	>40	No effects at this dose during a 21-day exposure period. Higher doses were not tried
Activators		
n-Propyl isome	>160	Death of one animal at this dose during a 90-day exposure period. Higher doses were not tried
Piperonyl butoxide	200	Based on a 90-day test
Van Dyke 264	>200	Symptoms but no deaths at this dose during a 90-day exposure period
Piperonyl cyclohexanone	100–200	Death of one animal at 100 mg. after 45 doses during a 90-day exposure period. No survivors after 4–10 doses at 200 mg.

to be slight. Single massive exposures to insecticides which are liquid at ordinary temperatures, or solutions of solid materials in kerosene or other petroleum distillates, must be considered as dangerous.

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Repeated daily skin exposure to insecticides, especially in solution, constitutes a real hazard. As a rule many of the materials in concentrated formulations are skin irritants which serves as a warning of dermal contact. However, in dilute solutions this warning sign is lost and because of the delayed effects of a number of insecticides, serious poisoning may occur during the handling and application before the danger of poisoning is realized.

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Biochemical Research Department

12 5094 THE DOW CHEMICAL COMPANY

Subject: RESULTS OF SKIN IRRITATION TESTS ON CURRENT PRODUCTION 2,4-DICHLORO-PHENOXYACETIC ACID AS COMPARED WITH PAST PRODUCTION MATERIAL.

File 123.14-11
Chg. 12-6-48
Rec'd 4-17-52
Fin'd 4-3-52
Work By R. L. Mellon-worth

To

Central Research Index
Executive Research Committee
Texas File A
Western Central Research File

H. R. Hoyle

Britton's Division
Attn: R. C. Dosser
C. A. Highhill

Medical Department
Attn: H. H. Gay

Safety Department
Attn: S. M. MacCutcheon

Agricultural Chemicals Division, 1-47 Bldg.
Attn: J. W. Britton

Check D.K.Rowe 4-11-52

Rept. By R.L.M. 4-10-52

MIDLAND VEAZEY OFFICE
APR 16 1952
INDEXED TYPED
CHECKED FILED
L.G.F. APR 17 1952

BCT 43.14-11-1

UNIT INDEX

The skin irritating properties of current production 2,4-D acid are similar to those of 2,4-D acid prepared by the new method introduced in 1950.

INDEX HEADINGS

2,4-Dichlorophenoxyacetic acid

Phenoxyacetic acid: 2,4-dichloro-,

Acetic acid: 2,4-dichlorophenoxy-,

Acetic acid: phenoxy-, 2,4-dichloro-,

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PROBLEM

A few cases of dermatitis have recently been observed in connection with the manufacture of 2,4-D acid by The Dow Chemical Company. A sample of current production material was submitted for skin irritation tests for comparison with past production material.

CONCLUSIONS

1. The skin irritating properties of current production 2,4-D acid are similar to those of 2,4-D acid prepared by the new method introduced in 1950.

2. Damp 2,4-dichlorophenoxyacetic acid is moderately irritating and strong solutions of the acid are slightly irritating to the skin on prolonged and repeated skin contact.

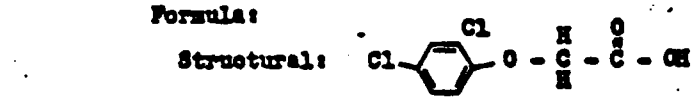
3. Dermatitis will probably result in some cases if the subject material, particularly if wet, is allowed to contact the skin repeatedly or for prolonged periods.

MATERIAL

Name: 2,4-Dichlorophenoxyacetic acid

Formula:

Structural:



Empirical: $C_8H_6O_3Cl_2$

B.R.D. File No.: T23.14-11-9

B.R.D. K No.: 2372-21

Source: Plant production

Sample obtained by: H. R. Hoyle

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EXPERIMENTAL RESULTS

When dry 2,4-D acid was bandaged repeatedly onto the shaven abdomen of a rabbit, five days per week, for a total of 10 applications in 14 days, very slight skin irritation of questionable significance resulted. When damp 2,4-D acid was tested in the same manner, moderate skin irritation characterized by hyperemia and superficial necrosis resulted.

Repeated applications of a 10% suspension of the material in water to the ear and shaven abdomen of a rabbit produced slight skin irritation. Repeated applications of a 1.0% suspension of 2,4-D acid in water to the ear and shaven abdomen of a rabbit produced very slight skin irritation on the abdomen.

DISCUSSION

Cases of dermatitis have been observed in connection with the manufacture of 2,4-dichlorophenoxyacetic acid in the past on a number of occasions. The present problem then in reality is an old problem.

A review of the conclusions reached in toxicological studies on 2,4-D acid reported by V. K. Rowe on 10-12-50 (Biochemical Research Department File Nos. T23.14-11-3 and T23.14-11-6) follows: 1. "It would seem from these results that impure 2,4-D is more likely to produce a significant amount of skin irritation than is pure material; and also, it seems that a damp product is more likely to produce irritation than is a dry one." 2. "Although neither the material prepared by the old or the new method is a skin sensitizer, the difference in ability to produce primary skin irritation is substantial and the likelihood of skin

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irritation developing in handling and use is much greater with the old product." These conclusions are substantiated and confirmed by the results of range finding toxicological tests on 2,4-D acid prepared by the new method reported by Richard Hollingsworth on 4-3-52 (Biochemical Research Department File No. T23.14-11-7).

Therefore, it is the considered opinion of the Toxicology Laboratory that the cases of dermatitis observed with current production 2,4-dichlorophenoxyacetic acid are very likely due to a difference in amount of skin contact with the 2,4-D acid rather than to any difference between current production material and 2,4-D acid prepared by the new method introduced in 1950.

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O. O. Fisher 9-27-52
Edwin E. Dunn 4-29-52

OFFICE COPY Biochemical Research Department

THE DOW CHEMICAL COMPANY

Subject: RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON 2,4-DICHLOROPHENOXYACETIC ACID PREPARED BY A NEW METHOD.

File T23.14-11-7
Chg. 5862
Rec'd 3-30-50
Fin'd 4-3-52
Work By R.L. Hollingsworth

To Check J. K. Rowe 4-11-52 Rept. By R. L. Hollingsworth 4-10-52

Central Research Index
Executive Research Committee
Texas File A
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Medical Department
Attn: H. H. Gay

Safety Department
Attn: S. M. MacCutcheon

+ 2,3,6-T (5)

UNIT INDEX

Results of range finding toxicological tests on 2,4-dichlorophenoxyacetic acid prepared by a new method are presented. Health hazards and precautions for safe handling are given.

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INDEX HEADINGS

~~2,4-Dichlorophenoxyacetic acid~~
~~Phenoxyacetic acids, 2,4-dichloro-~~
Acetic acid: (2,4-dichlorophenoxy-, toxicity)
~~Acetic acid, phenoxy-, 2,4-dichloro-~~

PROBLEM

A new method for preparing 2,4-dichlorophenoxyacetic acid was introduced in 1950 which gave a whiter and essentially odorless product. What are the toxicological properties of the subject material prepared by the new method of manufacture, and how do they compare with those of the older product?

CONCLUSIONS

1. 2,4-Dichlorophenoxyacetic acid is moderate in acute oral toxicity.

2. The free acid and its strong solutions are severely irritating to the eyes. Weak solutions are slightly irritating to the eyes.

3. The dry material does not produce significant skin irritation on repeated application to rabbit skin, but strong solutions produce slight skin irritation.

4. 2,4-Dichlorophenoxyacetic acid is not absorbed through the skin in any appreciable quantity.

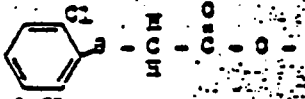
5. 2,4-Dichlorophenoxyacetic acid prepared by the new method is significantly less irritating to rabbit skin than the older product. Tests carried out on humans and reported by V. K. Rowe on 10-12-50 (Biochemical Research Department File No. T23,14-11-6) have confirmed the conclusions reached from studies on rabbits. Together these tests have shown that the

difference in ability of the material prepared by the old and new method to produce primary skin irritation is substantial and that the likelihood of skin irritation developing in handling and use is much greater with the old product.

MATERIAL

Name: 2,4-Dichlorophenoxyacetic acid

Formula:

Structural: 

Empirical: $C_8H_6O_3Cl_2$

Composition: 2,4-D 99.3%

B.R.D. File No.: 223.14-11-7

B.R.D. X No.: 2372-19

Freezing Point: 136.5°C.

Source: L. White, Britton's Division, 267 Bldg.

Reference: 234-122, 3-20-50

EXPERIMENTAL RESULTS

Acute Oral Toxicity

The compound was fed as a 10% solution in olive oil to groups of 5 female rats each by intubation. The results of the acute oral toxicity test are presented in Table 1.

Table 1 - Results of Acute Oral Toxicity Test

Dosage (g./kg.)	Mortality Ratio = No. Rats Dead / No. Rats Fed
0.25	0/5
0.30	1/5
0.35	4/5
0.40	5/5

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Eye Irritation

When 2,4-dichlorophenoxyacetic acid or a 10.0% solution of the acid in propylene glycol was placed in the eye of a rabbit severe eye irritation characterized by marked pain, conjunctivitis and corneal damage resulted. When a 1.0% solution of the material in propylene glycol was tested in a similar manner, slight, transient eye irritation resulted. Immediate washing of a treated eye with water reduced the amount of eye damage produced.

Skin Irritation

When the dry 2,4-D acid was bandaged repeatedly on the shaven abdomen of a rabbit, five days per week, for a total of 14 applications in 14 days, no significant skin irritation resulted. Repeated applications of a 10% solution of the material in butyl carbitol acetate to the ear and shaven abdomen of a rabbit produced slight skin irritation characterized by slight hyperemia, scaliness and hair loss.

Skin Absorption

In the skin irritation tests conducted, there was no evidence that 2,4-dichlorophenoxyacetic acid was absorbed through the skin in any appreciable quantity.

HANDLING HAZARDS

2,4-Dichlorophenoxyacetic acid and its strong solutions are severely irritating to the eyes.

The dry free acid apparently is not irritating to the intact skin; however, previous work on this compound reported on 10-12-50 by V. K. Rowe (Biochemical Research Department File No. T23,14-11-3) has shown the damp product to be slightly irritating to the skin on prolonged and repeated contact. Also strong solutions

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of this material produce slight skin irritation on prolonged and repeated skin contact. Accordingly, there is good reason to believe that prolonged and repeated skin contact by humans with the damp acid or its strong solutions might result in the development of dermatitis.

2,4-Dichlorophenoxyacetic acid is moderately toxic when swallowed. Although it would be possible for a person to consume a sufficient amount of the material to be dangerous to life, there is slight hazard from ingestion in ordinary industrial operations.

PRECAUTIONS FOR SAFE HANDLING

It is strongly recommended that eye protection be required whenever the free acid or its strong solutions are handled. Face shields, goggles with side shields, or the equivalent will afford suitable protection. Suitable facilities for washing the eyes with water should be readily available.

[redacted] precautions should be taken to prevent prolonged or repeated skin contact with 2,4-dichlorophenoxyacetic acid, especially with the damp acid or its strong solutions, such as might occur from wearing contaminated clothing [redacted] or from failure to wash the skin soon after contact with the material. Rubber gloves may be suitable for preventing contact on the hands and wrists.

WHAT TO DO FOR EXPOSURES

If 2,4-dichlorophenoxyacetic acid contacts the eyes, they should be washed thoroughly with flowing water for at least fifteen minutes, and then medical attention should be obtained as soon as possible.

In the event of contact upon the person, contaminated

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clothing or shoes should be removed and the skin should be washed thoroughly with soap and water. Medical attention should be obtained if skin irritation is apparent or if any pain persists. Contaminated clothing should be washed thoroughly before re-use.

If 2,4-dichlorophenoxyacetic acid should be swallowed, vomiting should be induced promptly by tickling the throat with a finger or by giving an emetic such as a soap solution or salt water (2 tablespoonfuls of table salt in a glass of warm water). Medical attention then should be obtained at once.

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Reprinted from Association of Food & Drug Officials of the United States
Vol. XVI, No. 2, April 1952

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CHEMICALS IN FOODS: A REPORT TO THE ASSOCIATION OF FOOD AND DRUG OFFICIALS ON CURRENT DEVELOPMENTS

PART II. PESTICIDES

SECTION III. SUBACUTE AND CHRONIC TOXICITY

ARNOLD J. LEHMAN, M.D.

Chief, Division of Pharmacology, U. S. Food and Drug Administration

Sections I and II in this series of articles on pesticides dealt with acute and dermal toxicity, and appeared in the October 1951 and January 1952 issues of the Bulletin, respectively. The present article summarizes the subacute and chronic toxicity of a number of important pesticides. Because of the time and effort involved in long-term studies, the list is not as extensive as that found in the first two articles. Table IV summarizes the subacute and chronic toxicity in rats. It will be understood from the wording of the table headings that between the lowest level with gross effects and the highest level without gross effects there were no intermediate dosage levels. Some special effects of the rodenticides in rats are presented in Table V, and Tables VI and VII summarize the highlights of chronic studies of certain heavy metals and chlorinated hydrocarbon insecticides in dogs. Some of these studies are still in progress and therefore certain conclusions in the present paper may be altered in the light of later data, and certain missing figures will eventually be supplied.

Identity of the compounds which appear in Section III will be found in the first article of this series.[1]

SUBACUTE AND CHRONIC TOXICITY IN RATS

This is summarized in Table IV. Any study of less than about a year must be classed as subacute. A point of interest in the tabulation is that the response of animals to chronic ingestion of a pesticide does not follow a set pattern. For example, in the feeding of rotenone there is a good parallelism between levels fed and degree of effect. The levels of feeding descend from a value which produces effects such as loss of appetite, retardation of growth and in some instances even symptoms of poisoning, to a lower value where no effects are observed, then to an even lower value at which only microscopic changes in tissues are seen, and finally to a value which produces no effects. There are some exceptions to the rule that the dietary level, at which effects as enumerated above are produced, is always higher than the level at which pathologic tissue changes make their appearance.

[1] Quarterly Bull. Assn. of F & D Officials of U.S., XV, No. 4, p. 122.

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TABLE IV

Subacute and Chronic Toxicity in the Rat

PESTICIDE	Time of Feeding in Weeks	DIETARY CONCENTRATION IN PARTS PER MILLION			
		Lowest Level With Gross Effects	Highest Level Without Gross Effects	Lowest Level With Tissue Damage	Highest Level Without Tissue Damage
Natural Products and Derivatives					
Rotenone	104	50	25	5	2
Pyrethrins	104	5000	1000	5000	1000
Allethrin	16	10,000	5000		
Ryania	16	15,000	10,000		15,000
Dihydrorotenone	16	100	25		
The Metals					
Copper (as chloride)	104	2500	1000	500	250
Mercuric (as acetate)	104	40	10	10	2.5
Mercuric (as phenyl mercuric acetate)	104	40	10	0.5	0.1
Selenium (from grain)	104	3*		3*	
Selenium (as selenide)	104	10*		10*	
Cadmium (as chloride)	104	45	15*	15*	
Antimony (as trioxide)	104	1000	500*	500*	
Barium (as chloride)	104	10,000	2000	10,000	2000
Chlorinated Hydrocarbons					
DDT	104	100	50	5	1
TDE	104	400	100*	100*	
DFDDT	28	1000	200*	200*	
Methoxychlor	104	200	100	500	200
ThH	104	100	50	50	10
ABH	104	100	50	50	10
BBH	104	10*		10*	
DBH	104	800*		800*	
Lindane	104	100	50	100	50
Toxaphene	104	400	100	100	25
Chlordane	104	75	25	2.5*	
Aldrin	16	50	25*	25*	
Dieldrin	16	50	25*	25*	
Prolan	40	200*		200*	
Bulan	40	1000	200*	200*	
Organic Phosphates					
Parathion	104	25	10		100
EPN	52		180	180	60
ASP-47	52	180	60		180
HETP	12		1000		
Triethyl phosphate	34		10,000	5000*	
Diethyl phosphate	20		5000		5000

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TABLE IV—*Continued*

PESTICIDE	Time of Feeding in Weeks	DIETARY CONCENTRATION IN PARTS PER MILLION			
		Lowest Level With Gross Effects	Highest Level Without Gross Effects	Lowest Level With Tissue Damage	Highest Level Without Tissue Damage
Dinitro Compounds					
DN-111	35	1000	200		1000
Herbicides					
2,4-D	30	5000	1000	1000*	
2,4,5-T	30	5000	1000	1000*	
Methyl-1-naphthalene acetic acid	104	20,000	2500	20,000	2500
Endothal	17	500*		5000	2500
Maleic hydrazide	17	50,000	10,000	50,000	10,000
Activators					
n-Propyl-isome	17	5000*		5000*	
Piperonyl butoxide	17	5000*		5000*	
Van Dyke 264	17	5000*		5000*	
Rodenticides					
1080	104	25	10	5*	
ANTU	104	100	50	50*	
Warfarin	1 to 2	6.25 (death)*			
Zinc Phosphide	5			100*	
Miscellaneous					
Arasan	65	1000	300		
Aramite	32	5000	1000	1000	200
Phygon	52	2500	1000	200*	
341 C	26	10,000	5000*	5000*	

* Lowest level fed.

Parathion is an example of this. It may be noted that no histopathological changes could be demonstrated in animals fed a diet containing 100 ppm. parathion for 2 years. This concentration is 4 times that which produced symptoms of poisoning, and 10 times the level at which no such effects were noted. Other compounds showing similar responses are methoxychlor and Endothal.

Another point of interest is that the LD_{50} of a pesticide is of very little if any significance for predicting its chronic effects. BBH may be cited as an example. In this instance the LD_{50} of BBH is approximately 6000

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mg/kg, a relatively low order of acute toxicity. However, the chronic ingestion of BBH leads to gross and microscopic evidence of intoxication at the low level of 10 ppm. in the diet, a high order of chronic toxicity.

A third point is that the behavior and general appearance of an animal chronically ingesting a pesticide is a crude endpoint as a measure of chronic toxicity. This is demonstrated with DDT. A dietary concentration of 100 ppm. is necessary before evidence of poisoning is observed, yet microscopic evidence of liver damage is seen at 5 ppm. The importance of histopathological examination of tissue of animals on long-term feeding studies cannot be overemphasized.

TOXICITY OF RODENTICIDES IN BAIT FORM

Table V presents a summary of the influence of food on the effectiveness of rodenticides. These pesticides are usually employed in the form of baited

TABLE V

Toxicity of Rodenticides

RODENTICIDE	LD_{50} IN MG./KG.	
	As the Chemical	In Bait Form
1080	1.7	5
Castrix	1.7	10
ANTU	6	30
Strychnine	16.2	25 (females) 158 (males)
Thallium sulfate	25	25
Zinc phosphide	450	450
Red squill	300	100–500 (females) 500–2000 (males)

food, hence, for the purpose of this experiment various dosage levels of each rodenticide were incorporated into 4 grams of laboratory diet and these 4-gram portions were offered to starved rats. Animals not promptly consuming the entire amount were rejected. The effect of the bait on the oral LD_{50} is summarized below.

A comparison of the values shows that the bait reduced the rodenticidal effect of all compounds except thallium sulfate and zinc phosphide. Warfarin was not tested in this manner because its effect is dependent on a continuous and prolonged insult to the coagulating mechanism of the blood and therefore not adaptable for study under the above conditions.

SUBACUTE TOXICITY OF SOME HEAVY METALS IN DOGS

The compounds listed in Table VI were fed to the animals 7 days a week, the prescribed dosage being mixed with a small portion of the diet before

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being administered. The daily dosage was calculated on the basis of the metal.

For the dog on a dry diet 1 mg/kg/day represents about 40 parts per million in the total diet. If each of the values in the above table is multiplied by 40 an approximate figure will be obtained representing the dietary concentration in parts per million. It should be pointed out that this table also indicates differences in toxic action of substances if given in small quantities of food (dogs) a..d in the bulk diet (rats). In addition true individual differences may play a part. By making use of some of the data in Table IV it is possible to compare the relative susceptibility of dogs and

TABLE VI

Tolerance of Dogs to Some Heavy Metals Administered Orally

METAL	DAILY DOSE	DURATION OF ADMINISTRATION	EFFECTS
	mg/kg.		
Arsenic (as trioxide)	3.18	60 weeks	No gross evidence of injury
Lead (as acetate)	0.33	33 weeks	Fatal to the animals
Cadmium (as chloride)	10.0	56 weeks	No gross evidence of injury
Barium (as chloride)	20.0	53 weeks	No gross evidence of injury
Selenium (as selenate)	2.5	4 weeks	Fatal to the animals
	0.5	60 weeks	No gross evidence of injury

rats to subacute poisoning by heavy metals. This may be summarized as follows:

METAL	PARTS PER MILLION IN THE DIET	
	Dog	Rat
Arsenic	127	217
Lead	less than 13	4000
Cadmium	400	45
Barium	800	2000
Selenium	20	between 3 and 10

This summary emphasizes the fact that in appraising the hazards of pesticides toxicological studies should include more than one species of animals.

CHRONIC TOXICITY OF CHLORINATED INSECTICIDES IN DOGS

In these experiments the animals were fed the insecticide daily 5 days a week. The insecticide was dissolved in corn oil and administered in capsule form, the one exception being an experiment with DDT in which the dry capsuled material was given. The results are summarized in Table VII.

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TABLE VII

Survival Time of Dogs Fed Various Daily Doses of Chlorinated Insecticides in Corn Oil

INSECTICIDE	DAILY DOSE	NUMBER OF ANIMALS	NUMBER OF DEATHS	EARLIEST DEATH	LAST DEATH	FATE OF SURVIVING ANIMALS
	mg./kg.			days	days	
DDT	80*	4	0			Sacrificed at 1100 days for autopsy
	80	10	10	23	300	
	50	3	1	190		Survivors sacrificed at 1185 and 1490 days for autopsy
	10	2	0			Sacrificed at 1490 days for autopsy
TDE	80	5	5	50	990	
	50	4	1	600		One animal sacrificed at 930 and two at 1410 days for autopsy
Methoxychlor	200	4	2	105	290	Survivors sacrificed at 1200 and 1350 days for autopsy
TBH	50	3	3	35	48	
Lindane	15	7	7	2	53	
	10	6	6	14	221	
Toxaphene	25	3	3	1		
	10	2	1	33		Survivor sacrificed at 1350 days for autopsy
	5	4	0			Sacrificed at 1360 days for autopsy
Chlordane	80	2	2	32	37	
	40	2	2	25	26	
	20	2	2	30	63	
	5	4	4	39	650	
Aldrin	5	2	2	21	22	
	2	2	2	24	178	
	1	2	2	109	344	
	0.5	3	0			One animal still living at 277 days and two at 180 days
Dieldrin	10	2	2	16	17	
	5	2	2	28	35	
	2	2	2	22	23	
	1	2	2	83	300	
	0.5	4	2	14	201	Two animals still living at 275 and 180 days, respectively
Heptachlor	5	2	2	13	21	
	1	4	3	265	424	One animal still living at 455 days

* Administered in the dry form.

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If use is made of the relationship that 1 mg/kg/day for the dog on a dry diet represents approximately 40 parts per million in the total diet, the dosages given in Table VII can be expressed in terms of parts per million and the susceptibility of dogs and rats to some of the chlorinated insecticides can be compared on a similar basis. One useful criterion in evaluating the chronic effects of a substance is the mortality rate of the various levels of feeding after 6 months on the diet. In case of the dog if our choice is limited to that dosage level at which some animals survived for at least 6 months and compared with similar data for the rat, the following values for the two species are obtained:

INSECTICIDE	SUSCEPTIBILITY RATIO IN PARTS PER MILLION IN THE DIET	
	Dog	Rat
DDT	2000	800
TDE	2000	2500
Methoxychlor	12,000	2000
Lindane	400	1600
Toxephene	400	1600
Chlordane	200	400
Aldrin	40	200
Dieldrin	20	200

This summary demonstrates the general tendency that dogs are more susceptible to the effects of poisons than rats, and again brings to mind the necessity for evaluating the pharmacological effects of a substance in more than one species of animals before attempting to assess the hazards.

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CHEMICALS IN FOODS: A REPORT TO THE ASSOCIATION OF FOOD AND DRUG OFFICIALS ON CURRENT DEVELOPMENTS

PART II. PESTICIDES

SECTION V. PATHOLOGY

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Sections I, II, III and IV of this series of articles dealt with the acute, dermal, subacute and chronic toxicities, and with certain biochemical aspects, of a number of important pesticides. The reports were published in the October 1951, and January, April and July 1952, issues of the Quarterly Bulletin, respectively. The present report gives the principal gross and histopathological changes as observed in rats following the subacute and chronic ingestion of pesticides. The pesticides discussed are those listed in Table IV in Section III of the series, and reference should be made to that table for the dosage necessary to produce some, but not necessarily all of the changes mentioned. Identity of the compounds listed in Table XV will be found in the first article of this series[1].

It should be emphasized that this tabular summary simply cannot go into details, does not consider the acute changes with massive dosages, and that much of the pathological study of pesticides done in the Division of Pharmacology is not represented in the table. Two of the major omissions are feeding studies in dogs with some of the compounds under discussion, and the inunction of them on rabbits. When the lesions observed in such studies have been strikingly different than those in the rat feeding tests, they will be briefly mentioned.

Generally speaking, the rat organs studied microscopically for each of the compounds listed in Table XV include lung, heart, liver, spleen, pancreas, stomach, small intestine, colon, kidney, adrenal, testis, ovary, uterus, thyroid, parathyroid, leg muscles, leg bones and bone marrow. The rat has no gall bladder. Urinary bladder, prostate and lymph nodes were sectioned less frequently than the other structures mentioned. It will be relatively obvious from inspection of the table that the liver is the organ most frequently affected, both microscopically and grossly, as might be expected from its detoxifying functions. On the other hand, although extrahepatic lesions occur less frequently, the sum total of such lesions shows a wide variety, and is an important part of the overall picture of pesticide toxicity.

[1] Quarterly Bull., Assn. of F&D Officials of U. S., XV No. 4, p. 122, (October) 1951.

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TABLE XV

The principal gross effects and histopathological changes in the rat following subacute and chronic ingestion of pesticides

PESTICIDE	GROSS EFFECTS	HISTOPATHOLOGICAL CHANGES
Natural Products and Derivatives		
Rotenone	Growth retardation; increased incidence of hepatic tumors	Peculiar. In survivors, a small number of hepatic cell masses ½ to 1 cm, between hyperplasia and tumor, at 2-10 ppm, but not at higher levels
Pyrethrins	Slight increase in kidney weight	Preliminary examination indicates that histological changes will be little if any
Allethrin	Growth retardation; tremors	Animals on this short-term experiment not examined histologically
Ryania	Growth retardation	Negative at 15,000 ppm
Dihydrorotenone	Growth retardation	Experiment in progress; only a few animals received as yet
Metals		
Copper (as chloride)	Mortality (5000 ppm); slight roughness of liver	Low grade necrotizing changes in liver and kidney; apparently increased incidence of lymphosarcoma and leukemia
Mercury (as mercuric acetate)	Growth retardation; kidney enlargement	Kidney damage
Mercury (as phenylmercuric acetate)	Growth retardation; kidneys enlarged and granular. Note: the values of 40 and 10 given in the first two columns under dietary concentrations in table IV, Section III, should have been 0.5 and 0.1 respectively	As above but at lower dosage levels. At highest level, occasional ulceration of cecum
Selenium (from grain)	Mortality; cirrhosis of liver; hemoperitoneum; ascites; hepatomas	Cirrhosis of liver; hepatomas; slight hyperplasia and hemosiderosis of splenic pulp; slight hyperplasia of bone marrow; slight focal myocardial fibrosis; minor renal changes

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Selenium (as selenide)	As above	As above
Cadmium (as chloride)	Anemia; bleached teeth	Hyperplasia of bone marrow and spleen; centrolobular degenerative changes in liver
Antimony (as trioxide)	Growth retardation; slight changes in color and texture of liver	Necrosis and other less severe degenerative changes in liver
Barium (as chloride)	Growth retardation	Slight degree of barium precipitation in urinary tract
Chlorinated Hydrocarbons		
DDT	Mortality (600 ppm); growth retardation; tremors; liver enlargement. (In dog, jaundice and hemorrhages.)	From all of this group of chlorinated compounds except Methoxychlor, the *liver* shows a more or less similar and characteristic complex of histological alterations. The complex consists of centrolobular hepatic cell enlargement, with increased oxyphilia, peripheral margination of basophilic granules, and (with formalin fixation) a tendency to hyalinization of the remainder of the cytoplasm. Qualitatively it parallels liver weight. At levels such as 600 ppm DDT or 200 ppm Chlordane the histological changes are outstanding and unmistakable, but at low levels the minimal changes present require experience and careful comparison with controls for detection. At the higher levels of feeding, non-characteristic changes such as focal necrosis, fatty degeneration, etc., may be mixed with the characteristic ones. The characteristic changes do not occur in non-rodent animals
TDE	Liver enlargement. (In dog, atrophy of adrenal cortex, and fatty liver.)	
DFDT	Liver enlargement. No bleaching of teeth.	
Methoxychlor	Growth retardation; liver tumors.	
TBH	Liver enlargement.	
ABH	Mortality (800 ppm); liver enlargement.	
BBH	800 ppm quickly fatal; liver enlargement.	
DBH	Liver enlargement.	
Lindane	Mortality (800 ppm); tremors; convulsions; liver enlargement.	Methoxychlor and to a lesser extent DDT caused slight increases in the incidence of hepatic cell adenomas. Chlordane caused a slight degree of adenomatoid hyperplasia of the hepatic cells
Toxaphene	Enlargement of liver and (slight) kidney.	
Chlordane	Mortality (400 ppm); liver enlargement; hyperexcitability.	
Aldrin	Mortality (100 ppm); convulsions.	Organs other than the liver are relatively uninvolved with this group of chlorinated insecticides. High levels of ABH, Lindane, TBH and Chlordane caused slight or moderate in-
Dieldrin	Mortality (100 ppm); convulsions.	

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TABLE XV—*Continued*

PESTICIDE	GROSS EFFECTS	HISTOPATHOLOGICAL CHANGES
	Chlorinated Hydrocarbons—*Continued*	
Prolan	Liver enlargement	creases in the amount of focal nephritis, and TBII caused moderate testicular atrophy
Bulan	Liver enlargement	It should be noted that not all of the compounds were fed for 2 years
	Organic Phosphates	
Parathion	Mortality (100 ppm); tremors	Long term animals negative. At maximum tolerated levels for 1 month, only changes of inanition
EPN	Some weight retardation at 180 ppm in females	At 180 ppm, in females, zona glomerulosa of adrenal cortex widened, with foamier cells
ASP-47	Mortality	Negative
HETP	Negative	Animals from this experiment not examined histologically
Triethyl phosphate	Negative	Slight hyaline granular degeneration of renal convoluted tubular epithelium, slight hyperplasia of bone marrow, slight increase in hepatic cell size
Diethyl phosphate	Negative	Negative
	Dinitro Compounds	
DN-111	Growth retardation	Negative at 1000 ppm; animals on 5000 ppm not examined
	Herbicides	
2,4-D	5000 ppm not tolerated; no effect from 1000 ppm	Questionable slight hypoplasia of bone marrow. None of the characteristic hepatic cell changes seen with the chlorinated insecticides
2,4,5-T	5000 ppm not tolerated; no effect from 1000 ppm	Negative. None of the characteristic hepatic cell changes seen with the chlorinated insecticides
Methyl-1-naphthalene acetic acid	Growth retardation	Examination not complete but it appears that there will be only minor changes

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Endothal	Growth retardation	Negative except for slight changes of inanition
Maleic hydrazide	Growth retardation	Negative
Activators		
n-Propyl isome Piperonyl butoxide Van Dyke 261	All showed liver enlargement of approximately the same degree	All showed about the same degree of periportal hepatic cell hypertrophy and slight fatty change, and of renal tubular pigment of a wear-and-tear type
Rodenticides		
1080	Mortality; bleached teeth; enlarged spleen; atrophic testes	Chronic congestion of spleen; atrophy of testis. Teeth not sectioned
ANTU	Mortality (200 ppm); growth retardation; spectacle eyes; thinning of hair; deformities of legs and feet	Hyperplasia of spleen, bone marrow and thyroid; thickening of spongiosa and thinning of cortex of long bones; hyaline centrolobular hepatic cells; calcified renal medullary tubular casts; terminal focal necrosis of stomach mucosa, thinning of adrenal cortex
Warfarin	Multiple hemorrhages	Multiple hemorrhages; questionable slight inflammatory changes in heart and liver
Zinc phosphide	Mortality. Liver damage (discoloration, nutmeg appearance, roughness of surface)	Fatty degeneration and necrosis of liver
Miscellaneous		
Arasan	Mortality (2500 ppm); disorientation; (unsteady on rear legs)	Preliminary examination indicates that histological changes will be slight, except for changes of inanition, in abdominal and thoracic viscera. At 2500 ppm, calcification in brain stem and cerebellum, and dystrophic changes in leg muscles
Aramite	Liver tumors and discoloration; growth retardation	Hepatic cell adenomas frequent at 5000 ppm; hyperplastic changes at 1000 ppm; 200 ppm not yet examined
Phygon	High mortality (2500 ppm); growth retardation	Possibly increased incidence of focal nephritis; 2500 ppm animals not examined
341 C	Growth retardation	Unusual. Negative except for foamy macrophages in lamina propria of villi of small intestine

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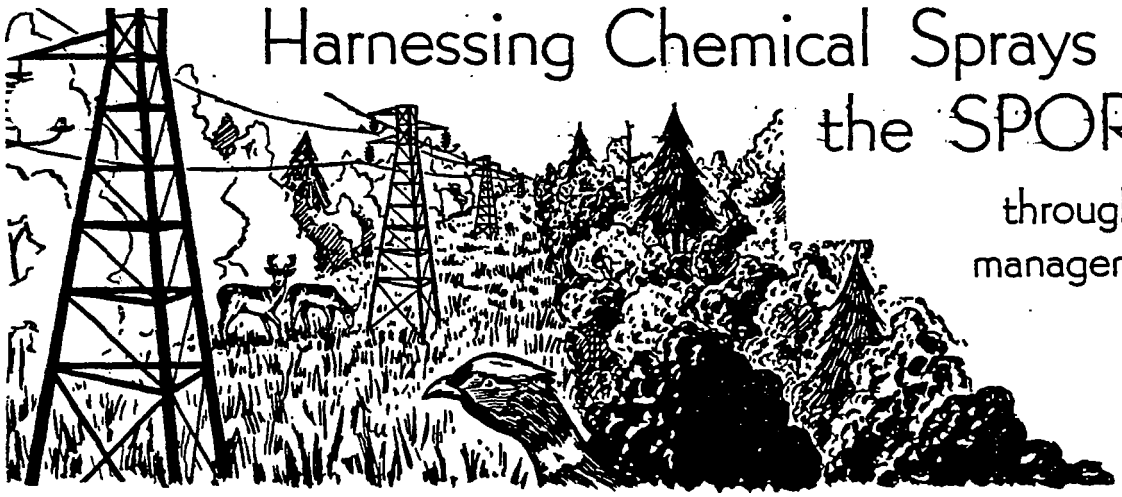
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Harnessing Chemical Sprays to Serve the SPORTSMAN

through new game management practices

Since the beginning of time man has looked at new ways of doing things with suspicion, sometimes justifiably. The American sportsman proved no exception when he first saw highway departments and power companies doing wholesale spraying of highway and utility right-of-ways to control brush with new chemical weedkillers. In many sections of the country, there has been an outburst of violent criticism of chemical vegetation control practices. The spoksemen for the sportsmen have claimed that such spraying destroys game, game habitat, flowers and natural beauty.

While there are certain real hazards connected with chemical weed control, properly used, most of the new weed killers can successfully be harnessed to serve the sportsmen through better game management practices without any significant evils.

It is true that all spraying should be done intelligently and under the supervision of trained, competent people. The manufacturers of weed control chemicals are doing their level best to see that their materials are judiciously applied. Like the sportsman, they condemn spraying which is done promiscuously and indiscriminately.

The sportsman need have no fear that maintenance men of highway departments and of America's power, telephone and telegraph companies are not alarmed when they hear reports of promiscuous spraying, because they are alarmed. They have much at stake. Chemical vegetation control means a savings of maintenance costs that adds up to millions of dollars. These savings are directly reflected in our utility rates in the case of power companies. Railroads and highway departments are effecting similar savings.

The American farmer was the first to make the use of chemical weedkillers standard field practice. In the grain states he increased phenomenally his output of small grains and corn by reducing weed competition. For the most part, his application of chemicals in crops has not brought objections from the sportsmen.

It has always been essential for farmers to cut brush in pastures and in drainage ditches. Like industrial users of spray materials, he was quick to discover the economies of chemical control. Most farmers are anxious to leave cover where it does not interfere with sound farming practice.

By selective use of brush killers woody species that benefit neither sportsmen nor farmers are being removed from fence rows to permit desirable species to spread or to be planted if they are not present.

In this new field of habitat control by chemicals the sportsman and farmer have surprisingly parallel interests. The program presents the sportsman with a new opportunity to better his public relations program with the farmer if he makes himself familiar with the farmer's problems and understands their relationship to game habitat.

The chemicals most widely used for vegetation control are 2,4-D and 2,4,5-T. Neither of these chemicals is toxic to man, animals, birds, fish or insects when used in accordance with recommended practices.

Actually, in the case of 2,4-D and 2,4,5-T dosages in excess of those commonly employed have been found to have no adverse effect on animal life. Tests have been conducted at several land grant colleges in which pastures were sprayed with common weedkillers, including 2,4-D and 2,4,5-T without adverse effect on cattle, horses, sheep, pigs, and chickens. In the laboratories of America's chemical industry and in the land grant colleges and in private testing laboratories these materials have been thoroughly studied by toxicologists. Wide experience over millions of acres has verified these experimental findings. For example, 2,4-D has been widely used for water hyacinth control on the bayous of Louisiana and in Florida without adverse effects on fish.

The hue and cry against indiscriminate spraying without any doubt started in instances where spray operators on

These men of West Penn Power Company crews are spraying a brush killer which is non toxic to wildlife. The relatively small strip of land they spray will be converted to grass by the second season. Over the years it will save the company's customers millions of dollars. Most conservation people recognize that this spray operation represents conversion of habitat rather than destruction of habitat.

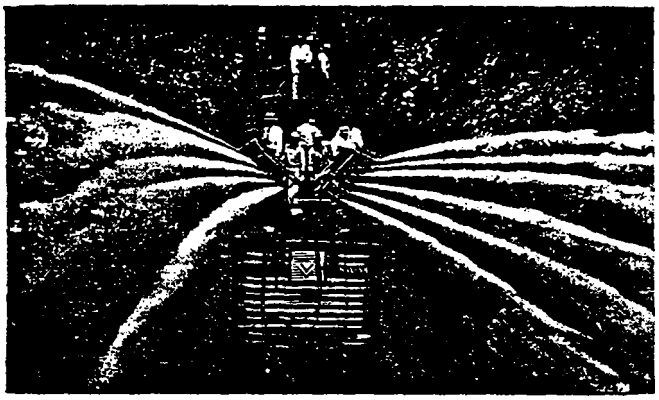


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highways attempted chemically to eradicate tall brush badly in need of cutting.

It is understandable that the sportsman who saw the unpleasant browned appearance that resulted from a few instances of such indiscriminate and ill advised spraying would well be concerned about the future of wildlife habitat.

Fears commonly expressed in relation to roadside spraying include the danger of erosion and the destruction of habitat. Most thinking game management men will agree



Railroads have a different problem than utility and telegraph companies in that they must remove brush for several yards from the track and all vegetation near the ties. This is a must for safe operation of our nations railroads. Obviously, grazing on railroad right-of-ways is not safe for animals, wild or domestic.

that the presence of brush along the roadside for game cover has been overemphasized. Such heavy cover is hazardous to the motorists, to the game, (when you consider fast moving traffic) and it sets up an ideal opportunity for the poacher who shoots from car windows.

Actually, there will still be adequate roadside cover for small game. Grasses grow profusely when brush is removed and more sunlight can reach the soil. Grasses are not damaged by 2,4-D or 2,4,5-T. Thick grass sod is one of the best covers for the prevention of erosion. In Oklahoma, soil conservationists have found 45% less run-off from a productive pasture previously sprayed to kill scrub oak than from adjacent mixed grass-brush land.

Dead brush along roadsides after chemical spraying is admittedly unsightly but it is a temporary factor in the process of chemical conversion from brush to grass. Spray crews know now that they must spray brush before it grows too tall or remove it by cutting. Stumps are then sprayed to prevent regrowth. Unless sprayed brush is tall, the increased growth of grass obliterates much of the dead brush.

The control of weed vegetation on right-of-ways is an integral part of maintenance. Proper use of selective herbicides can serve to convert game habitat rather than to destroy it. Where the outlook is conversion of habitat rather than destruction, game management authorities are beginning to see the value of selective herbicides as new tools.

In actual practice, some of the utility companies who are criticized by the sportsman when he first sees discolored brush, may in the long run pay for and provide improved habitat. The game specialists with whom the subject has been discussed are generally agreed that the grass land developed along lines by vegetation control is good for rabbits and deer.

2,4-D and 2,4,5-T are ideal for use in game management because of their very low toxicity to all animal life and the degree to which accurate application can be controlled. For example, it is possible to kill selectively susceptible species growing with more resistant species by using marginal concentrations.

The Forest Service has used these materials applied with hand sprayers to the base of large hardwood trees to liberate desirable pine plantings at a cost considerably below that of hand slashing. These same compounds are used to remove hardwood species from the more resistant Loblolly pines in the southeast.

In many areas under today's improved forest fire protection we have a condition in which wildlife species in need of grass and low-growing brush are finding uniform forest stands, such as aspen in the lake states, a very unfavorable environment. This is a recognized fact and, in areas where there are no desirable trees, game managers already have resorted to the use of the axe, controlled fire and even the bulldozer in an effort to open up the forest crown to permit growth of low brush and herbaceous material. When we consider costs of labor and mechanical bulldozing it is apparent that chemicals can compete very satisfactorily.

Judiciously used, chemical sprays can often do an even better and safer job at this lower cost. Consider the case of controlled burning — for game habitat improvement. Often on poor soils it is several years before any volume of ground vegetation reappears. In the meantime we have an erosion problem and quite possibly we jeopardize our public educational program for forest fire prevention. Using an aerial application it is possible to kill or injure the overstory of poor trees such as aspen and thus encourage the development of low growing shrubs and herbaceous plants. The plausibility of aerial application is proved by the past spraying of 500,000 acres of mesquite in the southwest at an average cost of approximately $5.00 per acre to improve carrying capacity of rangeland.

We do not wish to imply that selective herbicides will revolutionize game management practices. The need for interspersion of cover type is well recognized for such species as sharptail grouse, ruffed grouse, whitetail deer, cottontail rabbit, and other wildlife. Chemical sprays are just another tool available to professional game men in their effort to manipulate environmental factors to speed up production of today's hard hunted species. Like controlled burning and other established practices, indiscriminate use of herbicides could prove an enemy of wildlife. Properly employed, they can be useful tools in the hands of game management people. As new herbicides are developed and carefully tested it will become increasingly easy and profitable to selectively kill worthless plants while encouraging the type of cover that may be desired in any given area.

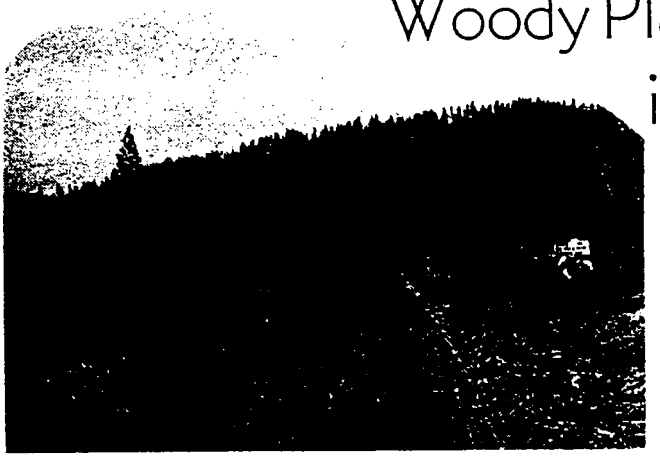
Farmers know what chemical maintenance can do to reduce weed population and taxes that are used to support our roads. Everywhere spray crews go there is widespread interest. This picture was taken in Ohio. Such programs can be conducted without injury to game or adjacent crops. Education and cooperation are the keys to success of such programs.



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Woody Plant Control Along Roads in Shasta National Forest

By ALVA G. NEUNS, *Training Assistant, California Forest and Range Experiment Station*[1]



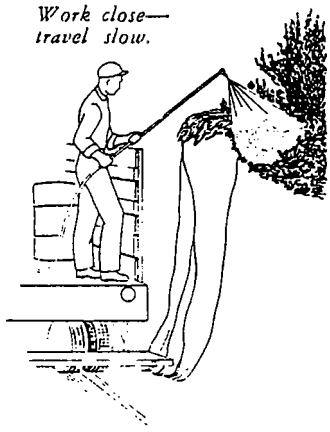
A mountain road in the Shasta National Forest mowed in 1949 and sprayed in 1950.

CONTROLLING sprouting brush species in the mountains with sprays of 2,4-D and 2,4,5-T depends mostly on how the chemical is applied. This has been demonstrated on the Shasta National Forest in California, where a fight is being waged against roadside brush with the battle cry:

100% COVERAGE — 100% CONTROL

One hundred and eighty miles of brush were sprayed in 1950 and 1951, and emphasizing the importance of total coverage accomplished three things. First — and most valuable — it gave something to achieve, a definite goal, so that "green" crews could be trained quickly and easily. Second, it assured complete kill of all sprouting growth above the ground and a maximum of root kill. Third, control was successful and long lasting enough that retreatment the next year was usually unnecessary. The last is important because of the inaccessibility of most forest roads.

Shasta National Forest has a network of 1200 miles of forest roads. They wind their way over steep mountainous country from an elevation of 1000 feet or less to 9000 feet. Some 900 miles of them have brush covered rights-of-way which in the past had to be hand-cut, bulldozed, or mowed with a brush mower every 3 to 5 years to keep the roads from disappearing beneath the brush.



Work close—travel slow.

In a search for less costly brush-control methods, the California Forest and Range Experiment Station tested the selective herbicides — 2,4-D and 2,4,5-T — from 1945 to 1949. Tests were made on plots growing more than 15 troublesome species, including live oaks, deciduous oaks, maples, manzanitas, chinquapin, cherry, snowbrush, and whitethorn. It was found that all of the above ground parts of these species could be completely killed and that the total root kill was high if complete coverage was achieved. Seedlings of all species could be completely killed.

Chemical spray carefully applied by research workers on plot-sized road shoulders is one thing, but the same formulation in the hands of the average spray man is quite another. Manpower shortages make it almost impossible to keep good foremen or spray men for any length of time once they are trained. Consequently, job operations and equipment were simplified as much as practicable. During the 1950 season, then, it was possible to spray 70 miles of previously cut, sprouting roadside brush with a high degree of success and at reasonable cost. The following rules for spraying and the slogan, "100% Coverage —100% Control," were based on the experience gained that year and are now used as a basis for all crew training.[2]

HOW TO SPRAY:

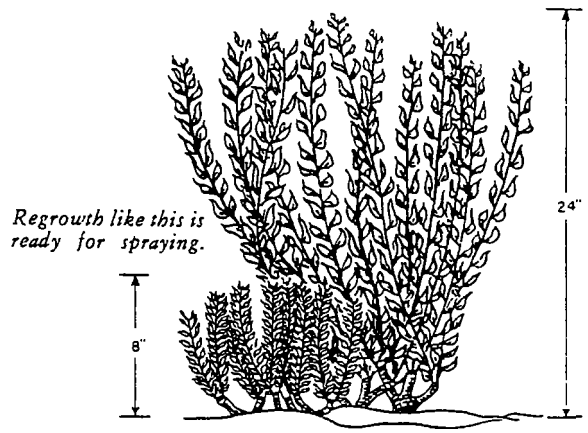
(1) Cover all leaf and stem surfaces to ground level with spray—especially the undersides of the leaves.

(2) Travel slowly so that you can *see* what you are spraying and know when the spray is covering the plant. This usually means walking rather than riding the truck.

(3) Hold the nozzle close to the shrub so that the spray-mist will not be blown away or wasted.

(4) Get down into dense growth using your short boom to pull it apart as you spray.

(5) Keep pump pressures high enough to ensure spray turbulence and misting.



Regrowth like this is ready for spraying.

(6) Check back after spraying from time to time to find what degree of coverage you are getting.

Rule (6) is very important. The spray-crew foreman should check back continuously to make sure all the growth is being treated. When diesel oil or an oil and water emul-

[1] The California Forest and Range Experiment Station is maintained by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of California, at Berkeley, Calif.

[2] The use of 2,4-D and 2,4,5-T for Brush Control on California Roads and Trails. U. S. Dept. of Agriculture. Forest Service, Berkeley, 1950.

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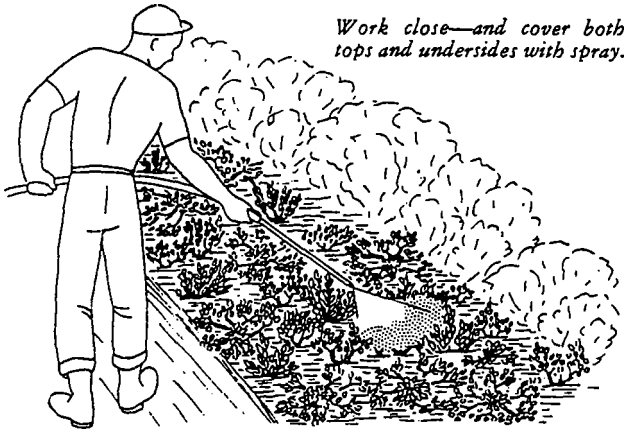
sion is used as a carrier for 2,4-D and 2,4,5-T, the spray material can be seen on the leaves and stems of the plants for several hours after the spray is applied. And nothing shows a spray-crew the importance of complete coverage more than to take them back over the job a week or two later when the leaves are drying. The men can see how much is still green when they don't get coverage — also how dead the plants look when they do.

Equipment used on the Shasta National Forest is simple, inexpensive, and readily converted for fire fighting and other purposes. It consists of a stakeside truck geared to travel very slowly and big enough to carry eight, 50-gallon drums—an average day's supply of spray mixture. A pump used in fire fighting that will develop up to 250 lbs. pressure (100 lbs. is minimum operating pressure) is mounted on a rear corner of the truck bed, 2 high-pressure hoses are attached and fitted with a short boom for average spraying and a longer boom for high banks. Trigger-type shut-off valves and solid cone nozzles (30° angle spray, 1.1 GPM at 100 psi discharge) on swivel fittings complete the assembly. Pumping is done directly from the 50-gallon drums. A low step is mounted on the back of the truck to carry spray men short distances or while spraying light brush.

This equipment is easy to use and requires little maintenance; 180 miles were sprayed continuously with no lost time due to equipment failure.

Walking speed and trigger control on the nozzles contribute as much to economy of spray as they do to total coverage. Complete control of the spray is a necessity wherever plant cover varies in density as it does on mountain road shoulders. Dense cover always requires continuous spraying, but medium and light densities do not. Intermittent spraying — shutting off the nozzle between plants—prevents waste of material on bare ground.

Average spray costs in 1950 on 70 miles of road, including labor, equipment and materials, were $28.00 per mile.



Work close—and cover both tops and undersides with spray.

Brush on all road shoulders had been cut and cleared for visibility and reduced fire hazard, with a brush mower or bulldozer, and the sprouting growth was treated with chemical spray. Some mechanical clearing costs as much as $240.00 per mile. Chemical control can eliminate the need for cutting in the future. Re-spraying will be necessary from time to time, but costs should decrease as control ecomes well established.

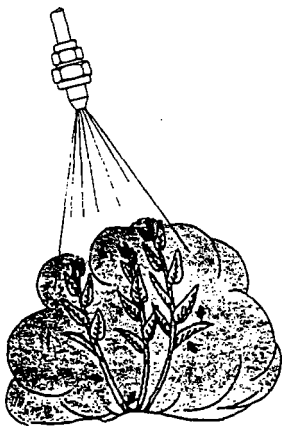
To decrease costs, oil and water emulsions were substituted for straight diesel oil as a carrier in 1951. Although the average cover was much denser in the area sprayed



The spray men walk unless there are very high banks to treat.

that year, costs on 110 miles of road were $25.00 per mile. As little as 4 gallons of oil per 50 gallons of emulsion were used, but oil was increased as the season advanced to insure better penetration of tougher leaves and stems.

During the 1950 field program an attempt was made to vary concentrations of 2,4-D and 2,4,5-T according to whether the predominating woody plant on the site was hard or easy to kill. Since spray material output averaged one 50-gallon drum per mile, it seemed possible to change the formulation in each new barrel to fit the hardest to kill species on the mile ahead. A table was provided from which the foreman could figure the number of quarts of each chemical needed per 50 gallons for a given concentration. Concentrations ranged from 8000 ppm 2,4,5-T and 4000 ppm 2,4-D to 1000 ppm of each for light retreatments. In 1951 to further simplify the job, concentrations were standardized at 4000 ppm 2,4,5-T and 2000 ppm 2,4-D. It was found to be easier to mix chemical, oil, emulsifier, and water at headquarters than depend on mixing and changing concentrations on the job. The slight advantage gained in root kill from higher concentration is offset by the loss of efficiency and labor time involved.



Spraying can begin as early as May in the lower altitudes and continue throughout September in the higher altitudes. The season of most rapid plant growth does not occur until late in July in the high country, so that a long spray season can be planned.

Woody plant species, exposure, stage of plant growth, soils, temperatures, moisture and growth rate all change mile by mile on forest roads. These and other factors are thought to influence the root killing power of 2,4-D and 2,4,5-T. But only one influencing factor can be made consistent throughout a mountain road brush-control job. That one factor is coverage, and a well-trained spray-crew and foreman who make total coverage a matter of pride can make the difference between poor control and 100 percent control.

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Controlling Scrub Aspen With BASAL SPRAYS

By JOHN L. AREND, *Research Forester*
Lower Peninsula Forest Research Center, Lake States Forest Experiment Station, East Lansing, Michigan

BASAL SPRAYS, also called "bark and dormant sprays," for controlling undesirable woody plants have been under study by various investigators in Michigan since 1946[1]. This paper describes the results of basal spray tests made three years ago which appear to offer a satisfactory method for controlling scrub aspen in northern lower Michigan.

THE CONTROL PROBLEM:

Aspen is a valuable tree species for wood products on good forest sites in the Lake States region. However, on poor sites aspen is usually an inferior grade tree. As a result, forest and game managers frequently need to control scrub aspen to improve wildlife habitat or establish tree species more adaptable to the forest site.

When aspen trees are cut or girdled mechanically, numerous sprouts and suckers develop from the stumps and roots for distances as far as 100 feet from the parent tree. Consequently, aspen reproduces itself very well after cutting. To date no satisfactory method for reducing the sprouting of scrub aspen has been reported.

Several investigators [2 3] have reported that the season of the year when aspen trees are cut has some effect on the vigor and number of sprouts and suckers which develop. Sprouts developing from aspen trees cut during the summer months have been found to be less numerous and less vigorous than those developing from trees cut at other seasons of the year. Aspen sprouts developing from trees cut in the summer, however, have generally been sufficient in number to satisfactorily restock the stand with aspen reproduction. Although the density and vigor of aspen sprouting can be reduced by summer cutting of the parent tree, the reduction is not sufficient for satisfactory control.

BASAL SPRAYS TESTED:

Starting in February 1950, the Lake States Forest Experiment Station initiated a number of chemical herbicide tests in northern lower Michigan designed to control several low value hardwood species, including scrub aspen, oak, and red maple, for pine release purposes. Different methods of chemical control including basal sprays, were applied to these low grade hardwoods in the winter, spring, summer and fall. Also, since aspen sprouting has been found to be somewhat affected by the season of the year when the parent trees are cut, a special seasonal study was made of scrub aspen treated with basal sprays at weekly intervals throughout the entire growing season (May through October).

The basal sprays tested included esters of 2,4-D (Esteron 44 and Esteron Ten-Ten), 2,4,5-T (Esteron 245), and 50/50 mixtures of each (Esteron Brush Killer) mixed with Grade 3 diesel oil. Concentrations of 2 and 4 percent by volume (8 to 16 lbs. of acid per 100 gals.) were applied to



Aspen sprouts and suckers which developed from aspen trees treated with basal sprays during the dormant and early growing season.

[1] At Dow Chemical Company, Midland, Michigan, and Michigan State College, East Lansing, Michigan.
[2] Zehngraff, P. J. 1946. Season cutting affects aspen sprouting. Lake States Forest Experiment Station Technical Note 250.
[3] Stoeckeler, J. H. 1948. The growth of quaking aspen as affected by soil properties and fire. Jour. of For. 46(10):727-737.

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the basal portion of individual trees to heights of 2 and 4 feet. In all cases the basal portion treated was carefully wetted to produce runoff completely around the tree.

EFFECTS OF BASAL SPRAYS ON TOPS OF SCRUB ASPEN:

All of these ester formulations of 2,4-D and 2,4,5-T when mixed with diesel oil at concentrations of 2 to 4 percent by volume and applied to the basal portion of aspen in *sufficient quantity* are capable of killing the top of the tree. The volume of herbicide applied in basal spray work is very important, especially for the larger trees. For example, only slight differences were observed in the rate of top kill and subsequent sprouting of aspen treated with 2 and 4 percent mixtures of esters of 2,4-D and 2,4,5-T in diesel oil when the basal portion of the trees were sprayed to a height of 4 feet. Moreover, a 2 percent mixture applied to the basal portion of the trees to a height of 4 feet is consistently more effective than a 4 percent mixture applied to a height of 2 feet. However, spraying the base of the tree to a height of 4 feet requires a considerable volume of herbicide compared to a 2 foot height.

When scrub aspen is treated with basal sprays (2 to 4 percent mixtures by volume of 2,4-D and 2,4,5-T esters in diesel oil) during the dormant season, the tops are generally dead by the end of the first growing season following the treatment. The treated trees usually leaf out in the spring, but the foliage starts turning yellow shortly after full leaf development. Scrub aspen treated with basal sprays during the early growing season starts showing the effects of treatment in about 10 days. However, trees treated in late July and August probably will not show the effects of the basal spray treatment until the following growing season. In other words, during the early growing season the tops of aspen trees are more easily killed by basal spray treatment than they are at any other season of the year. During the late growing season, and in the fall and winter months, aspen trees are generally more resistant to basal sprays. It is during these seasons that 2,4,5-T esters are more effective than 2,4-D esters. Also, a larger volume of herbicide needs to be applied to the basal portion of the tree during the fall and winter months than during the early growing season. Consequently, a 3 percent mixture by volume of Esteron 245 (2,4,5-T in diesel oil applied from the ground level to a height of 2 feet in soaking quantities is an effective year-around basal spray treatment for woody plant control.

EFFECTS OF BASAL SPRAYS ON SPROUTING OF ASPEN:

No sprouts or suckers were found at the end of the *first growing season* from the aspen trees treated with basal sprays at different seasons of the year. However, those cut and girdled sprouted prolifically the first year—the sprouts averaging about 3 feet in height. At the end of the *second* growing season suckers were found within 2 to 5 feet of the basal-sprayed aspen, *except for those treated after full leaf development. Three growing seasons* after the basal spray treatments the same sprouting condition prevailed —no suckers or sprouts were found from aspen treated after full leaf development through September, whereas suckers developed from the aspen similarly treated during the dormant period and during the early growing season before full leaf development.

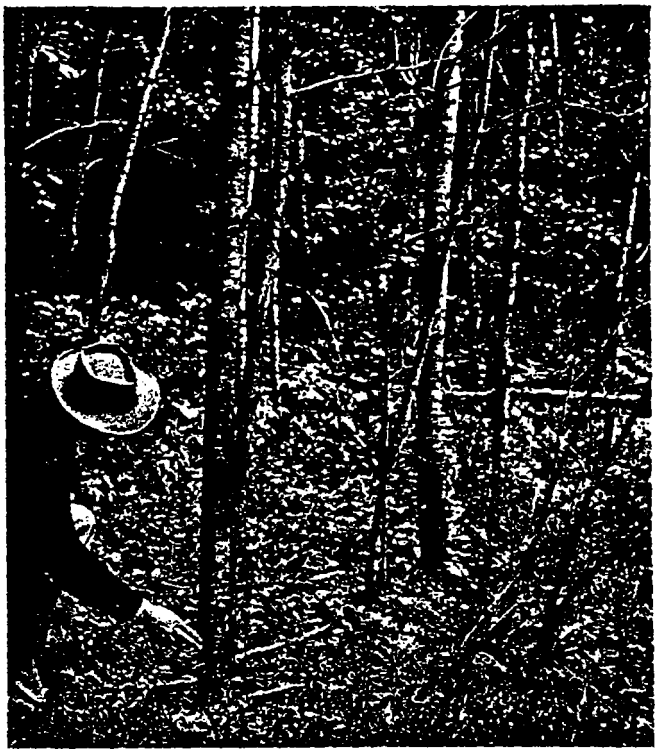
The weekly basal spray tests were conducted only on one area of scrub aspen. However, similar basal spray treatments were also applied to scrub aspen at two other locations in northern lower Michigan during the same year at various seasons: (1) dormant (February); (2) early growing season (May); (3) summer (July); and (4) fall (September). The sprouting results on the other test areas were the same, i. e., no sprouts or suckers were found among the scrub Aspen treated in July and only a few among the aspen trees treated in September. However, sprouts were both numerous and vigorous among the trees treated in February and in May.

SUMMARY AND CONCLUSIONS:

Scrub aspen trees have been killed without subsequent sprouting and suckering for three years in northern lower Michigan by applying basal sprays during the summer months of late June, July, and August following full leaf development. Aspen similarly treated during the dormant and early growing seasons suckered prolifically beginning the *second year* after the basal spray treatment.

Season of the year during which the scrub aspen is treated with basal spray has considerable effect on the concentration and volume of spray applied for an effective top kill as well as subsequent sprouting of the treated trees.

A 2 percent mixture (by volume) of 2,4-D and 2,4,5-T (Esteron Brush Killer) in diesel oil sprayed on the basal portion of the tree to a height of 2 to 4 feet is effective in killing the top of aspen during the growing season. However for year-around basal spray treatment, a 3 percent mixture of 2,4,5-T (Esteron 245) or 12 lbs. of acid equivalent per 100 gallons of Grade 3 diesel oil, applied to the basal portion of the tree to a height of 2 feet is recommended.



Aspen trees killed by basal sprays applied during late June, July and August after full leaf development. No sprouts or suckers have developed for three growing seasons after the trees were killed with basal sprays. (Trees sprayed summer, 1950. Photo taken in September, 1952).

Basal spraying aspen with either of these herbicide mixtures during late June, July and August following full leaf development offers a promising method of controlling scrub aspen without subsequent sprouting for at least three years.

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Botanist Oliver A. Leonard on the Davis Campus of the University of California points out 2,4-D filled cuts around the base of a large live oak tree. The cuts go an inch or two into the wood.

Chemical Control of Woody Plants in CALIFORNIA

By OLIVER A. LEONARD, *Associate Botanist, University of California, Davis, California*

Woody plants constitute problems on some of the following areas in California: (1) 2,500,000 acres of commercial timberland that is little more than brush; (2) 7,500,000 acres of woodland-grass; (3) 7,300,000 acres of chamise; (4) 2,400,000 acres of chaparral other than chamise; (5) 5,000,000 acres of big sagebrush. Woody plants, also, are problems on some other areas. A map is presented showing the distribution of some of the more important areas where brush is a problem in California.

There are many groups of people whose welfare is involved in controlling woody plants. Ranchers are interested in brush control in order both to reduce brush encroachment on cleared rangeland and to reclaim valueless brushland by transforming it into good pasture. They are interested in controlling brush and trees around springs and along water courses in order to have more water available for livestock and domestic purposes. In some areas, there is interest in controlling woody plants to make more water available for irrigation. Foresters are interested in woody plant control to aid in reforestation. Scotch broom (*Cytisus scoparius*) is a serious pest in the forested areas of several Sierra Nevada counties and prevents natural pine reforestation. It is readily killed by one application of 2,4-D (low-volatile ester) applied as a foliage spray in June or July, but seedling problems have not been solved. *Ribes* sp. must be controlled in the sugar pine (*Pinus lambertiana*) sites, since many members of this genus are alternate hosts for the white pine blister rust. Willows along stream banks hinder streambed maintenance and thus make flood control more difficult and complicate the mosquito control problem. Chemical brush control along rights-of-way is becoming more common. Poison oak (*Rhus diversiloba*) is of personal concern to more people in California than any other woody plant pest. It is controllable with either 2,4-D or 2,4,5-T, although 2,4,5-T is slightly superior. From one to three applications, spaced at yearly intervals, have been necessary for a complete control. There is some evidence, too, that acid or amine formulations, plus 1% low toxic spray oil, may result in better control than is obtained with some esters.

The Botany Department, University of California, Davis, California is conducting basic research on the chemical control of woody plants, including (1) cuticle penetration of herbicides and (2) radio-isotope studies. Working with live oak (*Quercus wislizenii*) and toyon (*Photinia arbutifolia*), for example, it was determined that 2,4-D moved rapidly out of treated leaves and thence downward in the bark in February when the shoots were still "dormant." Growth of roots during this period related both to the downward flow of materials from the leaves and to the fact that these woody plants are sensitive to hormone sprays at this time of the year.

Some general results of experimentation in chemical weed control in various types of woody vegetation are presented.

COASTAL SAGEBRUSH AND COYOTE BRUSH AREAS:

Coastal sagebrush (*Artemisia californica*) and coyote brush (*Baccharis pilularis*) are readily killed with 2,4-D at rates of 2 to 3 pounds per acre, applied by aircraft. According to available evidence, results should be more uniformly satisfactory when amine 2,4-D is used. The recommendation is 3 pounds acid equivalent per acre in about 8 gallons of water and one gallon of light-medium summer spray oil. The favored period for spraying is during April, May and June, but good results have been secured on coyote

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IN THIS ISSUE • *Chemical control of woody plants in California* • *A simple device for applying Esteron 245* • *Ethylene dibromide for the control of nematodes in the organic soils of Michigan* • *Dalapon, a new systemic grass killer introduced for control of industrial vegetation* • *Timber stand improvement on the W. G. Jones State Forest, Conroe, Texas* • *Report on Venezuelan experiments on grass control in rice with Premerge.*

• ON THE COVER •



See picture legend on page 3

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brush up to September 1. Two pounds of 2,4-D ester (low-volatile), plus 1 gallon of Diesel oil and 8½ gallons of water per acre have resulted in kills only slightly inferior to those obtained with the amine treatment.

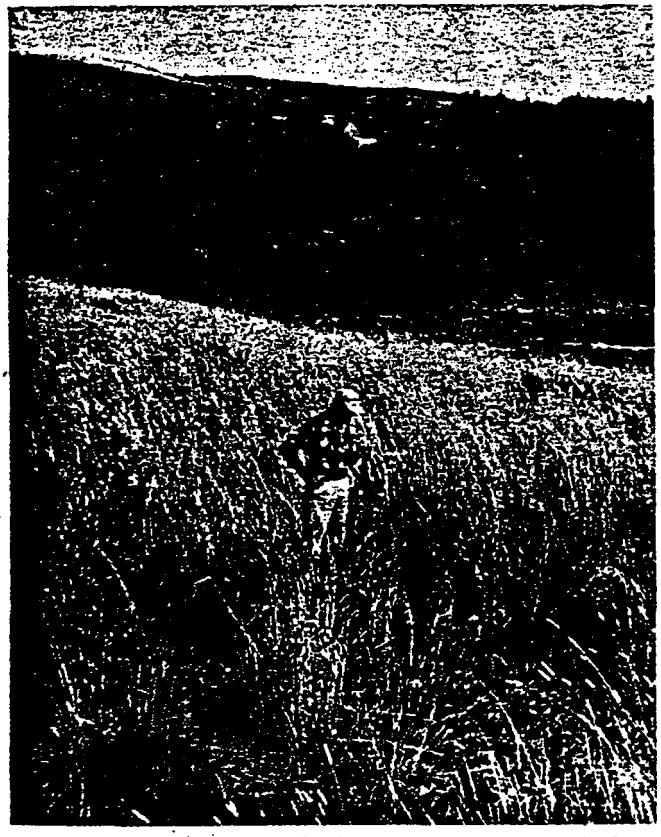
Control of mixed coastal brush with one application by aircraft is not adequate and must be followed by some additional spraying, probably with a ground rig. At present, the initial spray is with a brush killer using 2 pounds of acid equivalent per acre, plus 1 gallon of Diesel oil and 8½ gallons of water, applied by helicopter.

The advantages of spraying over other methods of brush control have been outstanding, especially in terms of increased grass production.

CHAPARRAL AND CHAMISE AREAS:

Because chaparral and chamise occupy so much land in California, considerable study has been undertaken to determine feasibility of control methods, the pattern of recovery from various treatments and the performance of desirable associated vegetation and subsequent seeding.

Chamise and chaparral have frequently been burned in the past, but these areas usually revert rapidly to the original condition because of crown sprouts and seedlings. The sites are frequently almost devoid of grass before being burned and killing the brush is of little value. Grass can be established most readily by burning the brush in the fall and seeding the desired grasses and legumes immediately. Grass competes with the brush sprouts and seedlings and results in the death of many of the latter. A broadcast spray should not be applied until the second spring after the burning and seeding so that (1) the legumes will have gone to seed once, building up a reserve of hard seed in the soil and (2) sprouts will have developed and most of the brush seeds will have germinated.



Chamise area seeded to perennial grasses. The author is standing on the boundary between a sprayed (background) and unsprayed (foreground) area. This is the same general area from which the data presented in the table were obtained.



Dr. Leonard is pointing to the location where 2,4-D amine has been placed in a cut in this interior live oak 33 months previously. The wood is rotting and stems have broken over. Eight separate cuts and 16 ml. of undiluted 2,4-D amine were used.

Good success has been achieved in killing brush sprouts and seedlings developing after a fire on chaparral and chamise areas by applying a broadcast spray, consisting of 2 pounds of low volatile 2,4-D per acre in 2 gallons of Diesel oil and 37½ gallons of water. March and April appear to be the optimum time for spraying. Such a spray will kill most of the chamise (*Adenostoma fasciculatum*), yerba santa (*Eriodictyon californicum*), golden fleece (*Haplopappus arborescens*), deer-weed (*Lotus scoparius*), rush rose (*Helianthemum scoparium*), creeping sage (*Salvia sonomensis*), and brush seedlings, including chamise, *Ceanothus sp.*, and *Arctostaphylos sp.* Seventy percent of the toyon (*Photinia arbutifolia*) may be killed. A partial top kill of interior live oak (*Quercus wislizenii*), coffeeberry (*Rhamnus californica*), and redbud (*Cercis occidentalis*) is secured. The latter species can be killed by from one to three applications of a spray containing 4 pounds acid equivalent of a brush killer in one gallon of Diesel oil and 98 gallons of water.

Where *Ceanothus sp.* are abundant, there is some advantage in using a spray that contains some 2,4,5-T, especially on the larger plants.

Aircraft spraying has not been sufficiently successful thus far to be used commercially.

Early in these studies, the isopropyl esters were compared with the mixed propylene glycol butyl ether esters of 2,4-D and 2,4,5-T, when formulated the same. One such test conducted in March 1951 resulted in a 22% and 20% kill of chamise sprouts with 2 pounds of the isopropyl esters of 2,4-D and 2,4,5-T, respectively, and a 78% and 54% kill with the propylene glycol butyl ether esters. The advantages in using some oil in the spray mixture have been demonstrated. There is some evidence to indicate that an oil of low toxicity gives a superior kill to that obtained with Diesel oil on chamise (high volume spraying).

There has been an increase in the production of grass on areas that have been sprayed, over that on unsprayed areas. One example of this was on a chamise site. The brush was broken down with a bulldozer in the summer of 1950 and the area burned in October of the same year. Immediately following the burning, the area was seeded by airplane to harding grass (*Phalaris tuberosa*), smilograss (*Oryzopsis miliacea*), tall fescue (*Festuca arundinacea*), and rose

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This helicopter (coming in for a landing) was used to spray coyote brush.

clover (*Trifolium hirtum*). Broadcast spray treatments were applied in 1952, (note table for one example). The quick returns as a result of spraying are indicated. It should be cautioned that the results obtained in seeding this perennial grass mixture were unusually good.

The effect of 2,4-D on chamise sprout kill and on grass production one year after spraying is shown in the table. A broadcast spray was applied on April 17, 1952, using the propylene glycol butyl ether ester of 2,4-D in 2 gallons of Diesel oil and 37½ gallons of water per acre. Grass production was determined in July 1953. (Coop. study with Charles Carlson, Calif. State Division of Forestry).

2,4-D per acre	*Kill of Chamise sprouts*	*Dry weight of grass per acre*
lbs.	%	lbs.
0	0	639
1	50	1378
2	87	1771

WOODLAND-GRASS AREAS:

There is considerable interest in clearing some of the woodland-grass areas. These areas are often burned, but a top kill is usually not obtained, except in live oak thickets or where brush has invaded. Bulldozing is commonly employed, especially where the soil and topography are suitable. After bulldozing and after fire, live oak sprouts develop from underground parts and grow rapidly, so that within a few years the area reverts again to the original state.

The present recommendation is to spray the sprouts with a mixture containing 4 lbs. of 2,4-D plus 2,4,5-T (Brush Killer), plus one gallon of Diesel oil plus 98 gallons of water. The spraying should be in the spring and early summer months and repeated until the sprouts are all killed. Very few sprouts will be alive after the third year.

The best kill of live oak sprouts thus far achieved by one application was with a spray containing 2 lbs. of 2,4-D (amine form) in 100 gallons of water plus 1 pint of a sticker spreader. The spray was applied in October of 1950 and by July of 1953, 56% of the stumps had no live sprouts left.

The cut-surface method using the amine of 2,4-D is applicable to the oak woodland areas. Cuts made with an axe or hatcher should be deep enough to extend well into the sap-wood. Trees are sensitive at all times of the year, but less chemical is required to kill trees in the winter and spring than in the summer and fall. On Digger pine (*Pinus sabiniana*), the cuts can be 8 inches (center to center) and good results obtained using 2 ml. of 2,4-D amine (4 lb. acid equivalent) per cut (undiluted). On Blue oak (*Quercus douglasii*), the cuts can be 6 inches center to center and again 2 ml. per cut should be used. Live oak is a vigorous sprouter and the cuts should be continuous for best results. Stumps can be treated the same as the standing trees, although some retreatment will be necessary; for example, from one to 6 sprouts had appeared on 4 out of 18 live oak stumps that had been treated 2 years previously with the amine of 2,4-D. 2,4,5-T amine appeared to be rather ineffective, since most of the stumps developed sprouts.

BIG SAGEBRUSH:

Sagebrush (*Artemisia tridentata*) is being controlled effectively with a mixture containing 2 lbs. of 2,4-D ester, plus ½ gallon of Diesel oil and 9 gallons of water, applied in June.

CONCLUSIONS:

Many different problems exist on the millions of acres of brush covered land in California. Chemicals are being used in many situations and their use will increase since certain jobs can be done better.

However, on much of the low-value rangeland, it will be necessary to do a good job more economically than is now possible. This is a challenge to industry as well as to federal and state research and extension agencies.



Distribution of woody plants in California (not including coniferous forests). A - big sagebrush. B - coyote brush. C - oak-woodlands. D - chamise and chaparral. E - coast sagebrush.

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A Simple Device for Applying Esteron 245

By DAVID B. COOK
Cooxrox Forest, Stephentown Center, New York

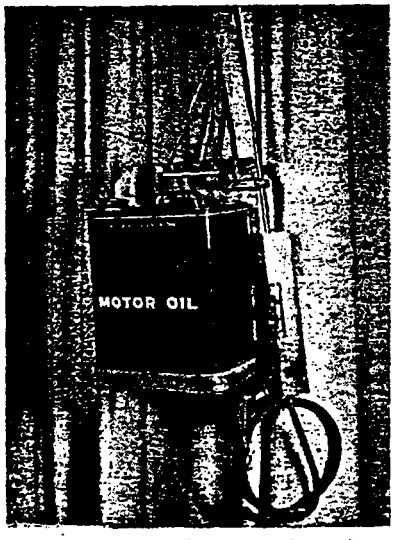
EDITOR'S NOTE: Mr. Cook reports on a home-made variation of basal bark application equipment. Two years' use has convinced him of its practical value. We are glad to publish his ideas which may prove helpful to others.

ESTERON 245 is rapidly making a name for itself as an effective silvicultural tool. Mixed with water, it can be used advantageously as a foliage spray on woody plants up to eight feet tall. Trees larger than that, and up to 6 inches in diameter at the ground, can best be treated with a basal spray of ESTERON 245 in oil. This latter technic is particularly useful in the selective deadening of unwanted, unmerchantable stems where a residual stand is to be retained. It is also a means of treating cut stumps to prevent sprouting.

The trade literature suggests that basal sprays be applied with a knapsack or small tank sprayer equipped with a wand bearing a nozzle set to deliver a narrow, solid cone of spray at low pressure. Such equipment is quite expensive. Moreover, it is impractical to use it for anything but "hormone" sprays because of the difficulty of completely cleaning it, and because "hormones" are effective against certain sensitive plants even in very small amounts. And, in practical operation, it is difficult to maintain an even, low pressure and to keep most of the spray on the target.

My early experience convinced me that such rigs were too complicated at least for my use; that pressure was unnecessary; and that simple gravity flow would be sufficient. It was apparent that a free flow of mixture would give a more thorough soaking effect and would run down onto the thin bark at the root crown better than would even the coarsest pressure spray.

A simple and effective basal sprayer was assembled from a two-gallon lubricating oil can with an air vent punched in the cap and a short pipe tapped into the bottom. For convenience in carrying, the can was mounted on a plywood packboard with rope shoulder straps. To the can was attached three feet of ⅜" "pure latex tubing", such as is used for handling blood. (I learned the hard way that ordinary red rubber tubing disintegrates from contact with kerosene). On this was threaded a clip-type tubing clamp and the end slipped onto a 24" length of ¼" aluminum pipe. Valve and aluminum pipe are mounted in a wooden handle to give a better hand hold. The whole rig weighs 3¼ pounds and can be put together for a little labor and about $1 in cash, the latex tubing being the most expensive item.



Basal spray applicator made with 2-gallon oil can.



The applicator in use. The drenching stream gives the stem a good soaking.

In operation, the tip of the wand is placed against the desired spot on the lower stem, the valve unclipped and the thumb pressure released enough to give the desired flow. The bore of the tube is ⅛", so the liquid flows out in a solid stream about the size of a match-stick, at no great pressure and with almost no wasteful spatter. Rate of flow can be nicely adjusted by thumb pressure.

On trees up to 2" in diameter, the kerosene will spread readily all the way around the stem from a single point of application. Somewhat bigger stems will require some lateral movement of the wand, while those over 4" must be spotted from opposite sides. In moving from tree to tree, the valve can be clipped shut; the small bore of the tube will keep the liquid from running out. When not in use, the wand is carried in the clothespin clip on the board, leaving the worker with both hands free.

This simple rig has several advantages. There being no pressure to maintain, the operator has one hand free and nothing to think about except aiming the wand. He has but to shrug his shoulders to find out from the sloshing how much liquid the can still contains. Filling is simply and quickly done. In two seasons of work, it has proved itself a good tool.

The formulation used has been a 4% solution of ESTERON 245 in kerosene, plus ½ teaspoon of Red-O oil dye; when freshly applied, this bright red is conspicuous, especially on grey birch. With this device, I have been able to accurately apply 2 gallons per hour, that being enough to treat about two hundred 3-4" trees. It is not so efficient on stems less than ½" in diameter because of the difficulty of holding the wand tip on such a small target, and letting out so small a quantity of liquid. The drenching stream gives the stem a thorough soaking, which may be one of the factors contributing to my uniformly good success in getting complete kill on a wide variety of northern hardwood species.

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TIMBER STAND IMPROVEMENT

On the W. G. Jones State Forest, Conroe, Texas

By CHARLES T. STEALEY, JR.*

In many areas of the south the once pure stands of southern pines have undergone changes, so that today the understory and in many cases the overstory is a mixture of southern pines and southern hardwoods. To help insure and increase survival, to enable more rapid growth and better development, to increase the maximum financial yields and to remove low valued hardwood competition from the higher valued intolerant pines, various individual treatments and combinations of treatments have been used in timber stand improvement programs. Several examples of individual treatments are girdling, frilling and felling. A combination treatment could be any of these individual treatments and the addition of a chemical herbicide.

The W. G. Jones State Forest, under the administration of the Texas Forest Service, is dedicated to demonstrational and research use. In an effort to obtain cost records and to set up a project as a demonstration of timber stand improvement and research with 2,4,5-T, a combination treatment of girdling and basal spraying with 2,4,5-T was used on a ninety-nine acre compartment of the forest.

The compartment treated was typical of many East Texas forest stands. The pine-hardwood overstory was composed of loblolly and shortleaf pine, several species of red and white oaks, sweet and black gum, mockernut hickory, American elm and dogwood, with pine making up sixty-five percent and hardwood thirty-five percent of the stand. The understory was composed of seedlings and saplings of hawthorns, bays and yaupon in a seventy percent hardwood—thirty percent pine—proportion.

The initial treatment consisted of girdling all hardwoods, eight inches d. b. h. and larger, which overtopped and/or interfered with established pine trees or reproduction, using the two hack girdling method. After the girdling was completed, the compartment was treated using the basal spray method. All hardwoods less than eight inches d. b. h. that overtopped and/or interfered with the pine were treated.

A two and eight-tenths percent concentration, by volume, was used for the basal spray, by mixing one and one-half gallons of Esteron 245 with fifty-three and one-half gallons of diesel oil. This concentration was selected on the basis of research work conducted by Ray E. Goddard, Assistant Silviculturist of the Texas Forest Service, who found satisfying killing results were obtainable with it, on the basis of preliminary work, and that it was more economical than the higher concentrations.

Spraying began in late March, 1952, and was completed in the latter part of April, using local unskilled labor. An inspection of the results of the treatment was made in November, 1952.

Table 1 is a summary of the cost study of the individual treatments and of the entire project.

The sizes of the trees girdled ranged from eight to eighteen inches d. b. h., with the average tree about twelve inches. The sizes of the stems sprayed ranged from less than one-half inch to eight inches, with the average about four inches. One gallon of the spray solution treated approximately seventy-five stems.

The number of stems treated per acre by girdling ranged from ten to thirty, with the average of about fifteen. The number of stems treated by spraying ranged from seventy-five to one hundred fifty, with the average of about one hundred ten per acre.

To check the results of the treatments, a cruise was made of the compartment in November 1952, dividing the treated stems into four degrees of results: dead, dying, abnormal and not affected.

Table 2 is a summary of the results of the treatments, showing the number and percentage of stems treated, the total number of stems and the cost per stem treated.

Tables 3 and 4 show the results of the individual girdling and spraying treatments.

The project from the standpoint of releasing established pines was very beneficial. A total of ten thousand four



Basal portion of sweet gum showing gum exudation from sprayed portion. The crown was just alive.

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Looking up at pine saplings and dead branches of overtopping hardwoods.

hundred ninety-four trees were released; seven thousand one hundred twenty-eight by spraying and three thousand three hundred sixty-six by girdling.

From the initial observations of this project it appears that the combination treatment gave more complete results in timber stand improvement than would either method by itself. Although the costs per acre for the individual treatments were lower than that of the combination, it only cost an additional one dollar and eighty-nine cents to release an additional seven thousand one hundred twenty-eight trees by spraying, after the compartment had been girdled. To girdle or lop this smaller material would have materially increased the girdling cost, not to mention the probability of sprouting, if no chemical was used. To spray the larger trees instead of girdling would increase the spraying cost materially above the cent and one-half per stem, hence higher costs per acre.

TABLE I

SUMMARY OF COST STUDY
TIMBER STAND IMPROVEMENT PROJECT

Item	*Hours*	*Cost*	*Cost/Acre*
Girdling labor	93.0	$ 69.75	
Girdling supervision	42.5	27.90	
Total girdling cost		$ 97.65	$ 0.99
Spraying labor	79.0	$ 59.25	
Spraying supervision	32.0	21.76	
Diesel oil (220 gal.)		28.16	
2,4,5-T (6 gal.)		78.60	
Total spraying cost		$187.77	$ 1.89
Total girdling cost		$ 97.65	
Total spraying cost		187.77	
Total cost		$285.42	$ 2.88

The results of this project show that over ten thousand pine trees were released at the nominal cost of two dollars eighty-eight cents per acre by using a combination treatment of girdling all hardwoods over eight inches d. b. h. and spraying all hardwoods under eight inches d. b. h. that were over-topping and/or interfering with established pine reproduction.

TABLE II

SUMMARY OF RESULTS OF TREATMENTS
TIMBER STAND IMPROVEMENT PROJECT

Item	*1 Acre*	*99 Acres*
Total hardwood stems	868	85,932
Total hardwood stems treated	136	15,444
Percent of hardwood stems treated	15.6	15.6
Total hardwood stems sprayed	121	11,979
Percent hardwood stems sprayed	13.9	13.9
Cost per stem sprayed	$.015	$.015
Total hardwood stems girdled	15	1,485
Percent hardwood stems girdled	1.7	1.7
Cost per stem girdled	$.065	$.065

TABLE III

SUMMARY OF SPRAYING TREATMENT
TIMBER STAND IMPROVEMENT PROJECT

Item	*1 Acre*	*99 Acres*
Total hardwood stems sprayed	121	11,979
Total dead	101	9,999
Percent dead	83.5	83.5
Total dying	5	495
Percent dying	4.1	4.1
Total abnormal	15	1,485
Percent abnormal	12.4	12.4
Total no effect	0	0
Percent no effect	0	0

1953 OBSERVATIONS OF RESULTS:

In the spring of 1953, several weeks after the vegetation had leafed out, an inspection was made of the results.

Many of the treated stems which had been reported as

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TABLE IV
SUMMARY OF GIRDLING TREATMENT
TIMBER STAND IMPROVEMENT PROJECT

Item	*1 Acre*	*99 Acres*
Total hardwood stems girdled	15	1,485
Total dead	14	1,386
Percent dead	93.3	93.3
Total dying	0	0
Percent dying	0	0
Total abnormal	1	99
Percent abnormal	6.7	6.7
Total no effect	0	0
Percent no effect	0	0

dead the previous fall had leafed out over one-half of the crown surface. A much smaller number had stump sprouted; had partially leafed out in the crown; or had leafed out from the stem. Over fifty percent of the stems treated showed no visible effects of the spraying whatsoever, and had seemingly recovered.

A late summer inspection of the compartment showed that many of the trees which had seemingly recovered at



Treating girdled hardwood with basal spray.

the time of the spring inspection, had died or were dying. Some few had recovered and showed little visible evidence of any spraying effects.

Sweet gum made up over ninety-five percent of the stems which had leafed out and had seemingly recovered in the spring and late summer inspections, and one hundred percent of the stems which showed no spraying effects in the late summer inspection. The red oaks made up the greatest proportion of the remaining five percent, with only minor percentages for all other species.

The following table shows a comparison of the results of the spraying treatment at the various inspection periods.

From the three observations of the results it appears that a low percentage concentration, by volume, of 2,4,5-T is sufficient to kill or seriously affect the overtopping smaller hardwoods in timber stand improvement work in southern pine-hardwood stands.

SUMMARY:

The results show that after eighteen months, over fifty



Pine sapling (foreground), dead sweet gum (immediately behind pine), and dead oak and sweet gum at left. Leaves are dead on tree at left.

TABLE V
COMPARISON OF SPRAYING RESULTS
TIMBER STAND IMPROVEMENT PROJECT

	PERCENTAGES		
Item	*Fall 1952*	*Spring 1953*	*Summer 1953*
Percentage dead	83.5	31.4	52.2
Percentage dying	4.1	1.6	38.2
Percentage abnormal	12.4	14.0	4.0
Percentage no effect	0	53.0	5.6

percent of the treated stems are dead, in many cases already fallen. Another thirty-eight to forty percent should be dead within a year. A portion of these stems may leaf out again next spring, but this summer's results show that only a relatively few will be able to survive and recover completely.

In the final analysis, if the results follow present indications, over ninety percent of the overtopping small hardwoods in southern pine-hardwood stands can be adequately controlled in a timber stand improvement program which is economical and easily accomplished and which produces the desired results.

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dustry as a fodder supplement for beef cattle, thereby improving meat quality and increasing quantity in less time with less food.

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Manuscript received February 11, 1953.

2,4-D Affects Phosphorus Metabolism

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A preliminary experiment in which *Commelina* sp. and *Xanthosoma* sp. were analyzed 24 hr, and 1 wk after being treated with 2,4-D (2,4-dichlorophenoxyacetic acid) showed that the percentage of water-soluble phosphorus in treated plants was consistently higher than in untreated plants. The following experiment was carried out to obtain additional information on the effect of 2,4-D on phosphorus metabolism.

A prepared field was divided into 12 plots each 52×24 ft and planted to a variety of white beans, Blanca Bonita (P.R. 1632). The experimental design consisted of 3 treatments each replicated 4 times in randomized blocks.

When the plants were about 15 in. high and had started to set fruit 1 plot in each of the 4 replications was sprayed with 0.1% aqueous solution of sodium 2,4-D. The plants in another plot of each replication were uprooted at the same time and left lying on the ground to die gradually. The third plot in each replication was left as a control. Two rows of unsprayed or undisturbed plants were left as borders around each plot. The treatments were started at 6 A.M., and samples of 100 plants were taken from each replication of all treatments at 4, 10, 24, and 48 hr and 1 wk after treatment.

The leaves, stems, and roots were separated, fresh and dry weights obtained, and a composite sample of 300 g of dry powdered tissue from each replication was analyzed for inorganic phosphorus. Aliquots of a hot water extract of the dry tissues were clarified with 0.5 g charcoal and used for inorganic phosphorus determination as described by Truog and Meyer (*1*).

Four hours after treatment the sprayed plants showed epinasty and other 2,4-D effects. Ten hours after treatment many of the leaves had curled, and the plants were becoming recumbent. The next morning the plants were somewhat chlorotic and the distortion had increased. The following day many of the leaves had developed necrotic spots. One week after treatment the leaves on most sprayed plants had withered and those that adhered were very chlorotic and sickly in appearance.

The uprooted plants remained fresh for the first day but after that they deteriorated so rapidly that by the end of the week it was not possible to obtain leaf samples.

Figure 1 shows the fluctuations of inorganic phosphorus in the leaves, stems, and roots, respectively, of treated, check, and uprooted plants.

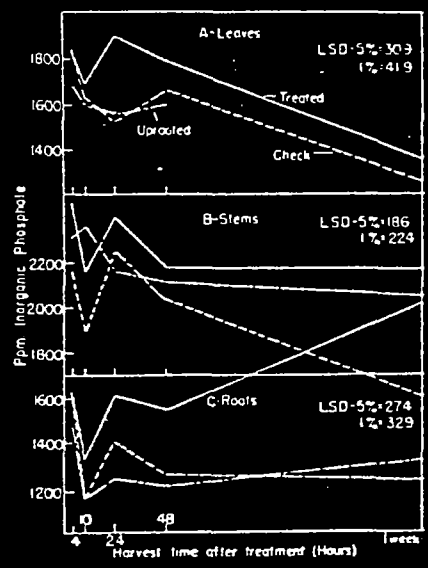


FIG. 1. Levels of inorganic phosphorus (ppm of dry matter) in bean plants analyzed at various intervals after treatment with 0.1% solution of sodium 2,4-D.

The data obtained at each sampling period, expressed as parts of inorganic phosphorus per million parts of dry material, was analyzed statistically by the analysis of variance and the least significant differences between treatments determined.

Four hours after treatment there was no appreciable difference in the amount of inorganic phosphorus in the leaves and roots of treated and check plants, but the stems of treated plants had a significantly higher amount than the checks. The uprooted plants had somewhat less inorganic P than the treated plants in all 3 organs. Ten hours after treatment inorganic P had dropped in all organs of all treatments except in the stems of uprooted plants, where it was somewhat higher; but in roots, stems, and leaves of treated plants it was higher and significantly more so in the roots and stems than in the corresponding organs of check plants. The samples taken 24 hr after treatment showed a sharp rise in the level of inorganic P in roots,

[1] Administered by the Office of Experiment Stations, Agricultural Research Administration, USDA.

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stems, and leaves of treated plants; the level in the roots and leaves was significantly higher than in the checks. There was also a rise in the inorganic phosphorus content of stems and roots of check plants, but it was less than in treated plants. The leaves of check plants showed no increase as did the leaves of treated plants in this phosphorus fraction, indicating that the most pronounced effect of 2,4-D on phosphorus metabolism occurred in the leaves at this time.

Forty-eight hours after treatment the level of inorganic P in leaves, stems, and particularly roots of treated plants was significantly higher than in check plants. In the roots, stems, and leaves of uprooted plants it was about the same as in the check plants. One week after treatment inorganic P in the roots of treated plants had increased significantly and this coincided with a sharp decline in the leaves, indicating that it may have been translocated from the leaves to the roots. There was practically no change in the inorganic phosphorus fraction in treated stems. Although by this time the level in the leaves and stems of check plants also declined, there was no corresponding increase in the check roots as there was in the treated roots.

Inorganic P in leaves and stems of treated plants fluctuated in most instances like that in the check plants, but this fraction was consistent, and at most sampling dates, except the first, significantly higher in roots, stems, and leaves of treated plants.

This experiment provides a clue to the mode of action of 2,4-D, e.g., it may inhibit or interrupt the phosphate metabolism in the plant. These data and the fact that very small amounts of 2,4-D produce drastic effects suggest that 2,4-D may inhibit or poison the enzyme or system responsible for the hydrolysis or synthesis of the high energy phosphates.

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Colorimetric Method for Determination of Aureomycin, Carbomycin, Erythromycin, and Terramycin in Aqueous Solution

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We have observed that the acid hydrolyzates of Aureomycin (*1*), carbomycin (*2*), erythromycin (*3*), and Terramycin (*4*) react with the arsenomolybdate reagent to produce blue colored complexes. The optical density of the color formed has been found to be a function of the quantity of antibiotic present. Satisfactory results have been obtained with the following procedure.

Aliquots containing from 10 to 40 μg of antibiotic are added to colorimeter tubes and the solution evaporated to dryness using an air jet. Two milliliters of 6*N* H_2SO_4 and 1 ml of arsenomolybdate reagent (Nelson's [5] reagent diluted with 2 parts of distilled water) are added. The tubes are plugged with loose fitting corks and placed in a boiling water bath. After a 15-min heating period the tubes are cooled to room temperature and the contents diluted with 5 ml of distilled water. Color intensity is determined using a photoelectric colorimeter equipped with 660 mμ filter. A series of tubes containing known quantities of the antibiotic are prepared and treated simultaneously with the tubes containing unknown quantities of the antibiotic. The values obtained with this series of known solution are used to calculate the constants of Beers' law and to standardize the determinations.

The sensitivity of the method varies somewhat with the particular antibiotic under consideration. The practical working range for Terramycin and Aureomycin is from 2 to 40 μg/tube; for erythromycin it is 5–80 μg/tube; and for carbomycin it is 10–160 μg/tube. Smaller quantities may be determined if only 1 ml of distilled water is added after the heating period, and micro cells are used to determine the optical densities. Apparently the hydrolysis with 6 *N* acid is necessary to obtain maximum sensitivity of the method, and use of more dilute acid resulted in reduced sensitivity. Only Terramycin will reduce the arsenomolybdate reagent without a preliminary hydrolysis, and in this instance the working range has been found to be from 20 to 160 μg/tube. Some of the data collected in analyzing aqueous solutions containing known quantities of Terramycin are summarized in Table 1.

TABLE 1

ANALYSIS OF SOLUTIONS FOR TERRAMYCIN CONTENT

Solution	Terramycin added* μg/ml	Terramycin found† μg/ml
Distilled water	0	0
	10	9.7; 9.9; 10.2
	30	29.8; 30.7; 30.7
	100	98.6; 99.7; 101.5
2% Glucose	0	0
	10	9.5; 9.7; 10.0
	30	27.7; 30.6; 30.6
2% Starch	0	0
	10	9.1; 9.7; 9.7
	30	28.8; 29.0; 29.6

* Terramycin hydrochloride was used in these studies. All analyses are presented in terms of the free base.

† Antibiotic extracted from aqueous solution with methyl isobutyl ketone.

This method cannot be applied directly to solutions containing carbohydrates and other substances which react when heated with the arsenomolybdate reagent. These four antibiotics may be separated from carbohydrates by extraction from aqueous solution (pH 7.2) into chloroform, amylacetate, *n*-butanol and methylisobutyl ketone. All the antibiotic has been recovered in the solvent phase when equal volumes of the solvent and aqueous solution have been used.

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Toxicity of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid

A Report on Their Acute and Chronic Toxicity in Dogs

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TOXICITY OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4,5-TRICHLOROPHENOXYACETIC ACID

A Report on Their Acute and Chronic Toxicity in Dogs

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BOTH 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) may act as plant hormones and herbicides [1] and are now being fairly widely used for the control of certain types of weeds on farm lands. They are being sprayed from airplanes on many acres of specialized farms and grazing land and are present in trademark preparations sold at retail for home gardeners. With the widespread use of these herbicides, possible toxic effects are important, as accidental ingestion may occur or residues may be inadvertently encountered in food. In an earlier report, 2,4-D was mentioned as being nontoxic to animals and man when administered orally.[1a,d] However, the injection of large doses of 2,4-D in animals produced symptoms similar to those seen in clinical myotonia.[2] More detailed observations were reported by Hill and Carlisle who studied the acute and subacute effects of 2,4-D in various species of animals.[3] There have been no reports concerning the toxic properties of 2,4,5-T. The present study concerns the acute oral toxicity and chronic oral toxicity of 2,4-D and 2,4,5-T in dogs.

EXPERIMENTAL PROCEDURES

Adult mongrel dogs of both sexes were used. Most of the animals were housed in the laboratory for a period of two to three months before the study was started. During this control period they were immunized against distemper with the Green vaccine. The dogs were fed a standard stock diet (Friskies) ad libitum. Both 2,4-D and 2,4,5-T were commercial materials, with a purity of 98.5 and 98.9% and freezing points of 132.8 and 150.6 C., respectively.

In the acute studies, the calculated dose of 2,4-D or 2,4,5-T was administered as a single oral dose in capsules. The dogs were observed for a period of 14 days, at which time the sur-

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vivors were autopsied. In the chronic studies, the 2,4-D or 2,4,5-T was administered orally in capsules five days a week over a 13 week period. Each capsule was imbedded in a 4 to 5 gm. piece of commercial canned dog food, which the animals consumed readily, thus avoiding the continued trauma of the stomach tube. During the study the animals were weighed twice a week. Control blood counts were taken before the administration of the drug, on the 30th day, and on the 90th day at the completion of the study. Hemoglobin was determined with a photoelectric colorimeter.[4] Upon death or at the completion of the study, the animals were autopsied. Tissues were taken from the lung, heart, liver, kidney, adrenals, spleen, thyroid, and ovary or testes, fixed in 10% formalin, and stained with hematoxylin and eosin.

TABLE 1.—*Effect of Single Oral Doses of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid in Dogs*

Dog No.*	Dose, Mg./Kg.	Weight Loss, Kg.	Symptoms†	Death Day‡	Mortality
		2,4-D			
1 F	400	−0.1	+	2	2/2
2 M	400	−0.8	++	3	
3 F	250	−1.8	+++	8	3/3
4 M	250	−2.0	++	7	
5 M	250	−0.4	++	4	
6 F	100	−2.5	+	8	2/4
7 F	100	−2.0	0	9	
8 F	100	−1.7	+	9	
9 F	100	−2.4	0	S	
10 F	25	0.0	0	S	0/3
11 F	25	+0.2	0	S	
12 M	25	+0.2	0	S	
		2,4,5-T			
13 M	400	−1.1	+	2	1/1
14 M	250	−1.4	+	3	1/1
15 F	100	−0.5	0	S	1/4
16 F	100	−1.0	0	7	
17 F	100	−1.0	0	S	
18 F	100	−0.4	0	S	
19 M	50	+0.3	0	S	0/4
20 M	50	−2.3	0	S	
21 F	50	+0.1	0	S	
22 M	50	−0.6	0	S	

* F in this column means female; M, male.
† Column indicates general severity of symptoms as discussed in text. 0 means no change from normal.
‡ S in this column means survived 14 day test period.

RESULTS

I. Acute Oral Toxicity.—(*a*) Mortality: The effect of single oral doses of 2,4-D or 2,4,5-T on survival for 14 days is shown in Table 1. The deaths were delayed and occurred two to nine days after the compounds were administered. The oral $L.D._{50}$ of 2,4-D was approximately 100 mg. per kilogram of body weight; for 2,4,5-T it was in the range of 100 mg./kg. or higher.

(*b*) Body Weight: The large oral doses of 2,4-D or 2,4,5-T produced a decrease in body weight (Table 1). Such animals developed various degrees of anorexia, and those that died refused even canned dog food toward the end of their survival.

4. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter with Light Filters, J. Biol. Chem. 115:63-75, 1936.

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(*c*) Symptoms: In dogs that died the effect produced by 2,4-D varied from a mild ataxia and stiffness in the hind legs to a definite myotonia. Initial signs were often noted six hours after the oral administration of 2,4-D. At this time the animals were more quiet than normal, and a slight ataxia was occasionally present. The hind legs were always affected first; the forelegs later or not at all. There was usually a progressive increase in spasm in the hind legs associated with increasing ataxia, and some dogs spontaneously extended the hind limbs in a spastic movement lasting a few seconds. The knee jerk was either normal or hyperactive.

In the early stages of acute poisoning with 2,4-D, the animals were usually quiet. If the animal was lifted and made to walk, the spasm and ataxia were clearly noticed, but after it had walked for a while, the spasm and ataxia tended to decrease. In the later stages of intoxication, the animal, when placed on its feet, was unable to stand and would then sit or lie in an awkward position with spastic limbs marked particularly by spasm of the hind legs. Other dogs stumbled and rolled on the floor in attempts to right themselves. An occasional dog showed evidence of irritation or pain when the skin over the back of the neck was grasped. Some dogs, when held up by the shoulders, would show extension and crossing of the hind limbs. As the toxic effects of the drug progressed, the dogs eventually refused the various types of food offered. Occasionally, sneezing, rubbing of the eyes, and diarrhea were observed, but not vomiting.

Such signs were also seen in one dog that survived a lower dose of 2,4-D for 14 days (Table 1). In this instance the changes were relatively mild and were limited to spasm of the hind legs and a slight ataxia.

Marked effects were not observed in the dogs receiving 2,4,5-T. The changes were limited to a slight or moderate stiffness in the hind legs, with the development of ataxia in the two dogs receiving the highest doses. No symptoms were evident in the one dog that died after a single dose of 100 mg./kg. (Table 1).

(*d*) Pathology: Changes observed were limited to the gastrointestinal tract and lungs. Four dogs (1, 2, 4, and 6) receiving 2,4-D and two dogs (13 and 17) fed 2,4,5-T showed mild to diffuse redness of the mucosa in the small intestine. In some dogs pneumonia was present. Histologically, the above dogs showed some necrosis and inflammation of the intestinal mucosa. One dog (13) had a moderate, diffuse hepatic necrosis. Two dogs (13 and 16) had a mild degree of renal tubular degeneration. In most dogs that died the findings were limited to nonspecific changes, such as hepatic congestion. In many cases death was apparently due to pneumonia, which followed the development of anorexia, weight loss, and myotonia.

II. Chronic Oral Toxicity.—(*a*) Mortality: All dogs receiving 2, 5, or 10 mg. of 2,4-D or 2,4,5-T per kilogram of body weight survived the 90-day test period. The dogs receiving 20 mg./kg. of these compounds died during the study. The administration of 20 mg./kg. in divided doses twice a day produced death in both dogs receiving 2,4,5-T and in one dog receiving 2,4-D (Table 2).

(*b*) Body Weight: The only significant change in body weight occurred in the animals that failed to survive the study (Table 2). Loss of weight in these dogs began 7 to 12 days before the death of the dog.

(*c*) General Symptoms: Dogs that survived the oral administration of 2,4-D or 2,4,5-T for 90 days were free of any symptoms.

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The three dogs that died while receiving the highest dose of 2,4-D showed signs differing somewhat from those observed in the acute studies. The dogs were quiet, weaker, and less responsive than normal. Muscle tonus was higher in the hind legs, particularly on passive extension. The hind legs were held more stiffly than usual when walking, and a slight ataxia was present. During the last two to three days of their survival two of the dogs showed difficulty in chewing or swallowing and eventually even refused a small bolus of canned dog food (usually readily consumed). There was also some oozing of blood from the gums and buccal mucosa.

TABLE 2.—*Body Weight and Survival of Dogs Fed 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid for 90 days*

Dog No.*	Dose, Mg./Kg.	Weight			Death Day‡
		Initial, Kg.	Final, Kg.	Diff., Kg.	
		2,4-D			
23 F	None	14.9	16.4	+2.7	S
24 F	None	9.3	10.9	+1.6	S
25 M	2	6.8	9.5	+2.7	S
26 F	2	15.2	13.8	−1.4	S
27 M	5	9.5	9.5	0.0	S
28 F	5	12.0	12.5	+0.5	S
29 M	10	14.7	14.8	+0.1	S
30 M	10	11.6	11.9	+0.3	S
31 M	10	17.2	17.8	+0.6	S
32 M	20†	13.0	13.7	+0.7	S
33 F	20†	9.8	5.9	−3.9	49
34 M	20	12.2	10.1	−2.1	18
35 M	20	13.4	9.7	−3.7	25
		2,4,5-T			
36 F	None	7.3	7.7	+0.4	S
37 M	None	12.1	14.5	+2.4	S
38 F	2	11.3	12.7	+1.4	S
39 M	2	8.9	10.0	+1.1	S
40 F	5	8.6	8.4	−0.2	S
41 M	5	12.3	12.5	+0.2	S
42 F	10	12.3	11.5	−0.8	S
43 M	10	13.0	12.2	−0.8	S
44 F	10	9.8	9.2	−0.6	S
45 M	20†	11.8	9.2	−2.6	49
46 F	20†	10.5	7.9	−2.6	75
47 F	20	14.9	12.7	−2.2	11
48 M	20	10.9	8.2	−2.7	59

* F in this column means female; M, male.
† Daily dose divided and administered one-half in a. m. and one-half in p. m.
‡ S in this column means survived 90 day test period.

In dogs that died while receiving 2,4,5-T, the prominent effects were weakness, slight stiffness in the hind legs, difficulty in swallowing food, and, in one dog, bleeding from the gums.

(*d*) Blood Count: The administration of 2,4-D or 2,4,5-T did not have any significant effect on the hemoglobin, red cell count, or total white cell count of animals that survived or died during the study. The differential blood count remained normal in the surviving animals. In three animals that died a terminal fall in the percentage of lymphocytes was observed (Table 3).

(*e*) Organ Weights: There was no significant change in the weight of the thyroid gland, adrenal gland, heart, liver, or kidney in animals that survived the 90-day period of study. In two of the three dogs that died during the administration

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of 2,4-D, there was a slight increase in heart and kidney weight. A similar change was noted in two of the four dogs that died as a result of 2,4,5-T.

(*f*) Gross Pathology: Two dogs receiving 2,4-D (26 and 34) showed areas of redness in the duodenum. With the administration of 2,4,5-T there developed in dog 42 a diffusely reddened duodenum and jejunum, while in dog 48, autopsied on the 59th day, a generalized icterus was demonstrated.

TABLE 3.—*Effect of 2,4-D and 2,4,5-T on Hemoglobin, Total Blood, and Differential Counts*

Dog No.*	Dose, Mg./Kg.	Day	Hb, Gm. %	RBC, Mil.	WBC	Differential Count: Neut., %	Lymph., %	Mon., %	Bas., %	Eosin., %	Death Day
					Control Dogs						
23 F	None	0	13.20	5.72	17,800	56	38	0	0	6	..
		30	13.82	5.93	15,600	64	29	0	0	7	..
		90	13.70	6.09	13,850	71	27	2	0	0	..
24 F	None	0	12.80	6.01	10,900	86	11	3	0	0	..
		30	14.72	6.66	13,150	79	12	7	0	2	..
		90	14.85	6.16	7,100	79	21	0	0	0	..
36 F	None	0	9.60	5.03	16,100	67	26	1	0	6	..
		30	10.75	5.63	13,300	56	16	2	0	3	..
		90	12.74	5.82	14,700	81	18	1	0	0	..
37 M	None	0	12.29	5.53	14,500	55	39	2	0	4	..
		30	13.31	5.78	14,900	63	26	4	0	7	..
		90	13.95	6.47	6,400	48	51	1	0	0	..
					2,4-D						
32 M	20†	0	10.43	5.02	13,800	64	27	5	0	4	..
		30	12.44	6.16	15,400	66	26	4	0	4	..
		90	11.52	6.35	12,100	70	26	6	0	0	..
33 F	20†	0	13.18	5.56	14,850	54	41	2	0	3	..
		30	14.46	5.88	9,700	47	46	4	0	3	..
		49	14.36	5.67	16,600	79	14	7	0	0	49
34 M	20	0	13.20	5.72	15,200	50	44	4	0	2	18
35 M	20	0	14.90	6.47	13,150	76	11	1	0	12	..
		25	14.59	6.34	9,100	68	27	4	0	1	25
					2,4,5-T						
45 M	20†	0	12.54	5.35	16,400	72	12	6	0	10	..
		30	8.61	3.96	16,450	77	13	3	0	7	..
		49	3.98	2.07	6,900	91	4	2	0	0	49
46 F	20†	0	12.48	7.10	16,050	63	29	2	0	6	..
		30	14.66	6.82	11,200	71	28	0	0	1	75
47 F	20	0	12.44	5.73	13,800	63	32	4	0	1	11
48 M	20	0	12.48	6.03	16,800	51	44	2	0	3	..
		30	12.93	5.81	14,650	76	18	4	0	0	..
		59	11.97	6.14	7,175	92	9	1	0	0	59

* F in this column means female; M, male.
† Daily dose divided and administered one-half in a. m. and one-half in p. m.
To conserve space, only data on control dogs and the highest doses are given.

(*g*) Microscopic Changes: The heart, lungs, thyroid, adrenal, ovary, or testes did not demonstrate any significant changes. Three of the dogs receiving 2,4,5-T and two receiving 2,4-D showed an occasional area of focal necrosis in the liver. This finding, however, was not related to either the dose of the compounds or the death of any animal (Table 4) and was judged to be of no significance. The duodenum of dogs 26, 34, 42, and 48 showed varying degrees of hyperemia and some early infiltration of cells in the mucosa. The submucosa and serosa were normal.

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A slight increase in the number of casts in kidney sections was noted in some dogs (Table 4), but again this was unrelated to dosage and was of doubtful significance.

COMMENT

In the acute studies, single large oral doses of 2,4-D administered to dogs may produce signs characteristic of clinical myotonia. This effect of 2,4-D was similar to that observed after various experimental species had received injections.[2,3] In addition, some dogs showed signs of irritation and pain when the skin of the back of the neck was grasped, which may indicate meningeal irritation. The extension and crossing of the hind limbs, when lifted by the shoulders, may point to a spinal or

TABLE 4.—*Histological Changes in Liver and Kidney of Dogs Fed 2,4-D or 2,4,5-T for 90 Days*

Dog No.	Dose, Mg./Kg.	Death Day	Liver		Kidney	
			Congestion	Focal Necrosis	Casts	Tub. Degen.
		2,4-D				
23	None	..	0	0	0	0
24	None	..	0	0	0	0
25	2	..	0	0	0	0
26	2	..	0	0	1+	0
27	5	..	1+	1+	0	0
28	5	..	2+	0	1+	1+
29	10	..	0	0	0	0
30	10	..	0	0	0	0
31	10	..	1+	0	0	0
32	20	..	0	0	0	0
33	20	49	1+	1+	0	0
35	20	25	2+	0	0	0
		2,4,5-T				
36	None	..	0	0	0	0
37	None	..	0	0	0	0
38	2	..	0	0	1+	0
39	2	..	0	0	0	0
40	5	..	0	0	0	0
41	5	..	0	1+*	2+	1+
42	10	..	2+	0	1+	0
43	10	..	0	0	0	0
44	10	..	1+	0	0	0
45	20	49	1+	1+	0	0
46	20	75	1+	1+	1+	0
48	20	59	2+	0	0	0

* Also a 1+ fibrosis.
Tissues graded on basis of 0, 1+, 2+, 3+, and 4+.

central lesion. Other effects occasionally observed were sneezing, rubbing of the eyes, and diarrhea. The effects of 2,4,5-T, previously studied, were not as severe, being limited to stiffness in the hind legs and ataxia. The higher doses of either compound produced anorexia and weight loss. Although initial effects may be seen six hours after the compounds are given, the syndrome developed slowly in the dogs, and death was delayed, occurring several days after the administration of the compounds.

In the chronic studies, the dogs survived doses of 2, 5, or 10 mg. of 2,4-D or 2,4,5-T per kilogram of body weight without apparent ill effects. However, the daily oral administration of 20 mg./kg. of 2,4-D produced death in three of four dogs within 18 to 49 days. This delayed death may indicate a cumulative effect of 2,4-D. The signs observed in animals dying from the 20 mg./kg. dose of 2,4-D

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differed somewhat from those seen in the acute studies. With the chronic administration, the animals exhibited chiefly a stiffness of the hind legs and ataxia, weakness, difficulty in chewing or swallowing, and occasionally bleeding from the gums. All four dogs which received 20 mg./kg. of 2,4,5-T succumbed between the 11th and the 75th day of the study. Toxic effects in these animals consistently included slight muscle spasm and difficulty in swallowing food. Thus, the chronic oral toxic dose for 2,4,5-T in dogs seems to be in the same range as that obtained with 2,4-D. With both drugs, there was a terminal loss in body weight.

Dogs surviving the chronic doses of 2,4-D or 2,4,5-T did not show any significant change in hemoglobin, total blood counts, or differential blood count. In three of the dogs (64, 57, and 105) which died while receiving 20 mg./kg. of 2,4-D or 2,4,5-T, there was a definite decrease in the percentage of lymphocytes in the peripheral blood. Hill and Carlisle [8] have observed a fall in the polymorphonuclear leucocyte count in one dog and a decrease in the per cent of lymphocytes in two dogs to which 50 mg./kg. of 2,4-D was administered intravenously each day for six days. In mice which received chronic injections there was no alteration in the peripheral blood picture.[2]

Large doses of 2,4-D administered intravenously or, in the present study, orally occasionally produced some necrosis in the liver. However, the administration of chronic oral doses produced only a slight and inconsistent focal necrosis of doubtful significance (Table 4). Deaths during the chronic oral administration of 2,4-D or 2,4,5-T were not correlated with significant lesions in the liver, kidney, or other organs examined.

SUMMARY

The acute oral $L.D._{50}$ of 2,4-dichlorophenoxyacetic acid (2,4-D) in dogs was approximately 100 mg. per kilogram of body weight. Doses in this range or higher produced a definite myotonia accompanied by anorexia and weight loss. The acute oral $L.D._{50}$ for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was in the range of 100 mg. per kilogram or higher. Such toxic doses produced only signs of a mild spasticity.

All dogs survived the oral feeding of 2, 5, or 10 mg. per kilogram of 2,4-D or 2,4,5-T 5 days a week for a period of 90 days. These doses did not produce any symptoms or changes in body weight, organ weights, or blood count.

Three of the four dogs receiving repeated doses of 20 mg. per kilogram of 2,4-D died, and all four dogs receiving 20 mg. per kilogram of 2,4,5-T died during the study. Toxicity to 2,4-D at the dosage level was accompanied by bleeding from the gums, necrotic changes in the buccal mucosa, and some difficulty in chewing and swallowing, but there was only little evidence of clinical myotonia. A terminal fall in the percentage of lymphocytes was observed in three of the animals.

Death during the repeated administration of 2,4-D or 2,4,5-T was not related to pathological changes in the liver, kidney, or other organs examined.

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SUSPECTED POISONING OF DOGS FROM EATING GRASSES TREATED WITH 2,4-D

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Frequently dogs are admitted to the veterinary clinic with the history that the owner feels that the animal has been poisoned from the effects of having consumed grass treated with 2,4-D. It has been commonly observed that dogs, on occasion, will eat various quantities of grass. We were unable to find any information in the literature concerning field cases of the toxicity of 2,4-D-treated grasses in dogs. Hill and Carlisle[1] have noted that, by intravenous daily administration of from 25 to 200 mg. of 2,4-D per Kg. of body weight, effects ranging from subacute intoxications to death may be produced. They further noted that dogs injected with 2,4-D showed a considerable susceptibility to the development of liver damage. Other work concerning 2,4-D poisoning in various animals has been done by other workers.[2-6] Since there is so little information available on 2,4-D poisoning in dogs, in which the 2,4-D was consumed on treated grasses or administered per os, the following trials were conducted.

Individual, heavy stands of lush growing bluegrass, orchard grass, timothy, and quack grass were sprayed with the water emulsion ester (butyl ester 4 lb./gal.) form of 2,4-D, at the rate of 4 pounds per acre. These are the most common grasses that dogs are most likely to eat. The spraying was at least twice as heavy as ordinarily recommended and sufficient to cause foliar damage to the grasses. The spraying was done on June 18, 1952, and the grasses were harvested in four separate lots (112 Gm. each) 48 hours later, on June 20, 1952. These grasses were chopped in small pieces and mixed in proportionately equal amounts with fresh horsemeat and dog meal. This mixture was fed to two healthy one-year-old mongrel dogs, weighing 20 to 25 pounds each, in equal amounts, in three consecutive feedings, two on June 20 at morning and evening meals, and the last one at the morning meal on June 21. Apparently the palatability of the food was not altered appreciably, as the dogs immediately consumed all of the food. The dogs were observed but no change in attitude or signs of any ill effects could be detected at any time within 96 hours following the first meal.

On June 24, both animals appeared to be in excellent health, and a subsequent trial was instigated. Each animal was given 5 cc. of the same ester concentrate of 2,4-D orally in a gelatin capsule. This dosage represents approximately 500 mg. of 2,4-D per Kg. It is noteworthy that this was a single dose given per os. No clinical deleterious effects were observed at any time within 96 hours following this oral administration.

On June 28, one dog was sacrificed. Necropsy examination of this animal failed to reveal any macroscopic lesions. The other animal, under daily observation, remained in apparently excellent health for the following 82 days, at which time these experiments were considered concluded.

We have not arrived at any definite conclusions with this meager information. However, in most instances of suspected 2,4-D poisoning entered at this clinic, some other cause has been definitely established. We are not cognizant of a proved field case of 2,4-D poisoning in dogs having occurred in this area.

Dr. Baker is associate professor of veterinary medicine, Dr. Ramsey is associate professor of veterinary pathology in the Division of Veterinary Medicine and Dr. Sylwester is extension professor of botany and plant pathology.

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CHEMICALS ARE EFFECTIVE
for Woody Plant Control in the South

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EDITOR'S NOTE:

The use of chemicals to increase forage and livestock production by the control of undesirable brush on range land and pastures is a relatively new and challenging field. The author is among the first to conduct an extension program which has brought such information to ranchers.

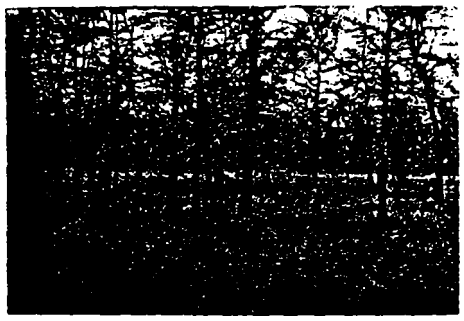
Woody plant control is one of the biggest problems faced by the farmer, ranchman and timber producer in the South today. There are about 240 million acres in the United States infested with undesirable brush and nearly half of this, or 108 million acres are in the fourteen southern states. With this brush under control, how much would it add to livestock producers' income?—possibly one-third of a billion dollars annually. Undesirable trees and brush use up water and minerals which should be available for forage growth for increased livestock production.

Some water resources information recently compiled by the Experiment Station in Texas illustrates this point. These data show that the annual average precipitation amounts to 361 million acre feet of which 131 million acre feet is lost to brush and weed growth, 44 million acre feet runs-off and only 30 million acre feet is used by desirable plant growth of all kinds. Actually, brush and weeds use one third of all precipitation received. We hear a lot about un-used run-off and find in Texas that three times as much water is utilized by worthless brush and weeds as runs off down our rivers. Actually, brush and weeds use *four* times as much water as all desirable plant growth—this includes all cultivated crops as well as range and pasture grasses. We have a potential of four times the present production in Texas if this water could be properly utilized. Lack of adequate water is not such a problem in all states but neither is the brush problem so immense. Texas has some 65 million acres infested.

Sometimes the brush infestation is so heavy that livestock handling on the range is difficult. Often this brush grows on the most productive land. Some brush controlled areas have produced five times as much grass as untreated ones the year following treatment even under drouth conditions. Average production increase following woody plant control usually varies from 20 to 50 per cent, depending upon rainfall, site and management practices. This means more pounds of meat per acre and increased efficiency of production. In timber areas, hardwoods often hold back or crowd out desirable pine trees. Control of these hardwoods increases the quantity and quality of forage production and also makes livestock handling easier. In forest areas the release of desirable timber growth is both practical and profitable.

Proper management following brush control is very important. Control of woody plants so that an operator can continue to overgraze is folly. Brush control is just one phase of a pasture management program just as building a tank or constructing a fence. For permanent benefits to result to a pasture, certain range management practices such as proper stocking, deferred or rotation grazing and in some cases reseeding and fertilization must be followed.

We are primarily concerned with chemical control of woody plants here and it does offer the most promise of any one method. Mechanical equipment such as bulldozers, anchor chains, brush and weed cutters and root plows often have a place in brush control and maintenance work. Sometimes a combination of mechanical and chemical methods will give the best control per dollar spent. Controlled burning in timber areas to increase grazing can be utilized in some cases. No one method of brush control can be recom-





Top Left: Post oak and blackjack oak aerially sprayed in May 1952, shows 63 percent root kill 18 months following treatment. Forage production has been materially increased. (Texas Agri. Expt. Sta. photo).

Lower Left: Cut-off stumps treated with 2,4,5-T in diesel oil did not sprout. Good native grass made a quick come-back.

Right: The author examines the trunks of post oak trees basal sprayed with 2,4,5-T in oil two years before. On six inch trees and larger, frill treatment is cheaper and is very effective.



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receiving 2,4,5-T were slight muscle spasm, and difficulty in swallowing. Histological studies revealed mild changes in the liver and kidneys.

The first experimental toxicological work conducted on farm animals was that of Mitchell and co-workers.[4] These investigators pastured sheep and cows on foliage sprayed with more than recommended amounts of 2,4-D without effect. They also fed a lactating cow 3.5 Gm. of 2,4-D daily for 106 days without ill effects. Milk from the cow did not contain a demonstrable quantity of 2,4-D. No effects were observed in the calf to which this milk was fed, nor was there any 2,4-D found in the serum of the calf. The serum of the cow, however, contained 8.4 p.p.m. of 2,4-D though none was found in the liver, kidney, or fatty tissues.

Early in 1950, Grigsby and Farwell[5] published the results of experiments with 2,4-D and 2,4,5-T involving domestic livestock. In this study, alfalfa was sprayed with various herbicidal formulations including three different 2,4-D preparations and one 2,4,5-T preparation. Livestock including horses, dairy and beef cattle, sheep, swine, and chickens were immediately pastured in the freshly treated areas. In summary of this work, the authors stated, "Results of this experiment indicate that none of the herbicides used had any serious physiological effect upon the livestock involved. They also indicate that none of the livestock preferred any of the sprayed areas to those that were unsprayed; however, with the lots sprayed with 2,4-D herbicides, the livestock grazed the areas sprayed almost as well as the unsprayed. Since the rates used were two to four times greater than recommended dosage, it seems that the farm use of these materials for pasture weed control is a reasonably safe procedure."

TABLE 1—Materials Studied*

Common name	Chemical name
2,4-D	2,4-Dichlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
MCP	2-Methyl-4-chlorophenoxyacetic acid or 4-Chloro-o-toloxyacetic acid
silvex	2-(2,4,5-Trichlorophenoxy)-propionic acid

* These are basic chemicals used in herbicidal formulations.

Materials Studied

Commercial herbicides generally can be described as formulations of active ingredients. The active ingredient usually appears as a salt which is soluble in water or as an ester which is soluble in oil. Formulations may be designed for use as water solutions, water emulsions, oil solutions, oil emulsions with water, or dusts, depending upon the use for which they are intended. Basically, most formulations contain, besides the active ingredients, a dispersant, a solvent, a wetting agent, and perhaps a diluent. These are usually considered to be inert ingredients although they may have a profound influence upon herbicidal effectiveness.

In the studies reported herein, toxicological data are reported not only for the basic active ingredients and their chemical derivatives (table 1) but also for the finished formulations as they are marketed* (table 2).

Oral Administration of Single Doses

Procedure.—The toxicity of the various herbicidal materials and formulations when administered in single oral doses to various species of animals has been determined. All rats, guinea pigs, and rabbits used were young adult animals from the stock colonies of this laboratory. The mice were young adults obtained from Carworth Farms. The chicks used were New Hampshire Reds obtained from a commercial hatchery when about 3 days of age and were about 3 weeks of age when treated.

The test materials were administered by intubation unless otherwise indicated. Aqueous solutions were used wherever possible. Olive oil or corn oil solutions were used where water was not appropriate. Surviving animals were observed until recovery was certain, usually about two weeks.

Results.—The results of the acute oral studies are summarized in tables 3 and 4.

* The trade-named drugs listed in table 2 and in the text are products of The Dow Chemical Co., Midland, Mich.

TABLE 2—Herbicidal Formulations Studied

Trade name	Active ingredients (%)
2,4-Dow weed killer (formula 40)	65.0 Alkanolamine salts of 2,4-D
Esteron 44	44.0 Isopropyl ester of 2,4-D
Esteron 245 (old formulation)	33.3 Isopropyl ester of 2,4,5-T; 13.1 Mixed amyl esters of 2,4,5-T
Esteron 245 (present formulation)	65.3 Mono-, di-, tripropylene glycol butyl ether esters of 2,4,5-T
Esteron ten-ten	70.5 Mono-, di-, tripropylene glycol butyl ether esters of 2,4-D
Brush killer 50-50	27.2 Butyl esters of 2,4-D; 26.5 Butyl esters of 2,4,5-T
Brush killer T	52.3 Butyl esters of 2,4,5-T
Esteron 76 (used in oil solution only)	37.1 Isopropyl esters of 2,4-D; 39.6 n-Butyl esters of 2,4-D
Esteron 76E (used in either oil solution or water emulsion)	36.8 Isopropyl esters of 2,4-D; 39.3 Butyl esters of 2,4-D
Esteron brush killer (old formulation)	25.6 Isopropyl esters of 2,4-D; 24.4 Isopropyl esters of 2,4,5-T
Esteron brush killer (present formulation)	34.3 Mono-, di-, tripropylene glycol butyl ether esters of 2,4-D 33.0 Mono-, di-, tripropylene glycol butyl ether esters of 2,4,5-T
Kuron (was called H-1078)	64.5 Mono-, di-, tripropylene glycol butyl ether esters of silvex
Dow MCP amine weed killer	69.1 Alkanolamine salts of MCP

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In all cases where the data were sufficiently extensive to allow statistical analysis, the l.d.$_{50}$ values with their 19/20 confidence limits were determined by the method of Litchfield and Wilcoxon.[7] In all other cases, the l.d.$_{50}$ values were estimated and the range between the dose allowing survival of all animals treated and the dose causing all animals to die is given. For comparative purposes, some of the val-

TABLE 3—Summary of Acute Oral Toxicity of Various Basic Herbicidal Materials

Material	Species	Sex	Vehicle	l.d.$_{50}$ (19/20 confidence limits) (mg./kg.)
2,4-D (2,4-Dichlorophenoxyacetic acid)	Rats	M	Olive oil	375 (302-465)
	Mice	M	Olive oil	368 (312-434)
	Guinea pigs	M and F	Olive oil	469 (397-553)
	Chicks	M and F	Olive oil	541 (358-817)
	Dogs (4)		Capsule	100 Range (25-250)
2,4-D, alkanolamine salts	Chicks (3)		Water (dosage on acid equivalent basis)	..... Range (380-765)
2,4-D, sodium salt	Rats	F	Water	805 (610-1,063)
	Rats (2)		Water	666 .....
	Guinea pigs	M	Water	551 (417-727)
	Guinea pigs (2)		Water	1,000 .....
	Mice (2)		Water	375 .....
	Rabbits (2)		Water	800 .....
2,4-D, isopropyl ester	Rats	M and F	Olive oil	700 (569-861)
	Guinea pigs	M	Olive oil	550 (451-671)
	Mice	M	Olive oil	541 (398-736)
	Chicks	M and F	Olive oil	1,420 (1,127-1,789)
2,4-D, mixed butyl esters	Rats	F	Corn oil	620 (320-954)
	Guinea pigs	F	Corn oil	848 (604-1,190)
	Rabbits	F	Corn oil	424 (252-712) Range
	Mice	F	Corn oil	712 (500-1,000)
	Chicks	M and F	Undiluted	2,000 (1,250-2,960)
2,4-D, mono-, di-, tripropylene glycol butyl ether esters	Rats	F	Corn oil	870 (510-640)
2,4,5-T (2,4,5-Trichlorophenoxyacetic acid)	Rats	M	Olive oil	500 (391-640)
	Mice	M	Olive oil	389 (245-619)
	Guinea pigs	M and F	Olive oil	381 (307-472)
	Chicks	M and F	Olive oil	310 (211-456) Range
	Dogs (4)		Capsule	100 (50-250)
2,4,5-T, isopropyl ester	Rats	M and F	Olive oil	495 (420-584)
	Guinea pigs	F	Olive oil	449 (362-557)
	Mice	F	Olive oil	551 (380-799)
2,4,5-T, mixed butyl esters	Rat	F	Corn oil	481 (312-739) Range
	Rabbit	M	Corn oil	712 (500-1,000)
	Mice	F	Corn oil	940 (674-1,312) Range
	Guinea pigs	F	Corn oil	750 (500-1,000) Range
2,4,5-T, mixed amyl esters	Rats	F	Olive oil	750 (500-1,000) Range
MCP (4-chloro-o-toloxyacetic acid or 2-Methyl-4-chlorophenoxyacetic acid)	Rat	M	Corn oil	700 (500-1,000) Range
MCP, amine salt	Rat	M	Water	1,200 (1,000-2,000) Range
	Guinea pigs	M	Water	1,200 (630-2,000)
silvex, (2[2,4,5-trichlorophenoxy] propionic acid)	Rat	M and F	Corn oil	650 (560-760) Range
silvex, mixed butyl esters	Rat	F	Corn oil	600 (250-1,000) Range
	Rabbits	F	Undiluted	750 (500-1,000)
	Chicks	M and F	Corn oil	1,190 (707-2,000)
silvex, mono-, di-, tripropylene glycol butyl ether esters	Rat	F	Corn oil	621 (473-814) Range
	Guinea pigs	M	Corn oil	1,250 (500-2,000) Range
	Mice	F	Corn oil	1,410 (1,000-2,000)
	Chicks	M and F	Corn oil	1,190 (847-1,670)
	Rabbits	F	Undiluted	819 (610-1,070)

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ues given in the literature are included in table 3.

Toxic symptoms generally observed in animals made ill with this type of compound include loss of appetite, loss of weight, depression, roughness of coat, general tenseness, and muscular weakness particularly of the posterior quarters. Postmortem findings usually include irritation of the stomach of small animals and of the abomasum of ruminants, minor evidence of liver and kidney injury, and in some instances congestion of the lungs.

ORAL ADMINISTRATION OF REPEATED DOSES OF 2,4-D TO RATS AND CHICKS

Experiment 1, Rats.—Procedure.—Matched groups of 5 or 6 young adult female rats from the stock colony of this laboratory were fed 2,4-D five times a week for four weeks by intubation. The dosages employed were 0.0 (controls), 3.0, 10.0, 30.0, 100.0, and 300.0 mg./kg. administered as aliquots of olive oil solutions emulsified in about 2 ml. of 5 to 10 per cent aqueous gum arabic solution. The controls received appropriate doses of olive oil.

Results.—The control animals and those animals receiving 3.0, 10.0, and 30.0 mg./kg. of 2,4-D showed no adverse effects as judged by gross appearance and behavior, mortality, growth, hematological values, blood urea-nitrogen concentrations, organ weights, and gross and microscopic examination of the tissues.

Rats receiving 100.0 mg./kg. showed varying degrees of gastrointestinal irritation, slight cloudy swelling in the liver, and a depressed growth rate. Those receiving 300.0 mg./kg. failed rapidly and died. Severe gastrointestinal irritation was the principal adverse effect observed.

Experiment 2, Rats.—Procedure.—Matched groups of 5 young adult female rats from the stock colonies of this laboratory were placed on diets containing 0 (control), 100, 300, 1,000, 3,000, and 10,000 p.p.m. (parts per million by weight) of 2,4-D.

Results.—The controls and those animals receiving the diets containing 100 and 300 p.p.m. of 2,4-D for 113 days showed no adverse effects as judged by food consumption, growth, general appearance, mortality, blood urea-nitrogen concentrations, hematological examinations, organ weights, and gross and microscopic examination of the tissues.

Those on the diet containing 1,000 p.p.m. for 113 days suffered slight adverse effects characterized by depressed growth rate, excessive mortality, slightly increased liver weights, and slight cloudy swelling of the liver.

Those on the two higher diets were destroyed after twelve days as they were not eating and were rapidly losing weight. Examination revealed increased liver and kidney weights and slight pathological changes in these organs.

Experiment 3, Chicks.—Procedure.—Twelve New Hampshire Red chicks weighing from 210 to 340 Gm. each were divided into four groups of 3 chicks each and maintained for seven days on diets containing 0 (controls), 500, 1,000, and 3,000 p.p.m. of 2,4-D.

Results.—The only adverse effects apparent grossly were a reduction in food intake and a retarded growth rate in the birds fed the diet containing 3,000 p.p.m. of 2,4-D. No histopathological examinations were made.

ORAL ADMINISTRATION TO STEERS

Purpose.—Since essentially all of the toxicological information on 2,4-D and 2,4,5-T type herbicides was obtained using small animals and since the practical application of these materials involves exposure also to large animals, experiments were undertaken to ascertain if extrapolation of the data obtained on the laboratory animals to large animals was justified.

Esteron Brush Killer.—Since esteron brush killer (formulation of esters of 2,4-D and 2,4,5-T) is perhaps the widest used of the products discussed herein, it was chosen for study.

In the experiments outlined in the following paragraphs, administrations were all by intubation. Treated animals were constantly under observation and the ordinary clinical observations were routinely made. In general, only positive observations are reported.

Experiment 1.—A steer weighing 291 kg. was given a single dose of 1,000 mg./kg. No perceptible symptoms of toxicity were observed.

Experiment 2.—A steer weighing 295 kg. was given 1,000 mg./kg. of esteron brush killer on each of three successive days. General depression, decreased food and water intake, and decreased rumen motility were observed following the third dose. These symptoms became increasingly severe until the steer died on the third day following the last dose. Death was undramatic; no convulsions, struggling, or signs of pain were observed at any time. Necropsy revealed the following: Rumen contents were dry and [illegible] strongly of the

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esteron: the abomasum was impacted and the contents of the intestines were entirely fluid; the mesenteric vessels were congested and the spleen was dark and shrunken.

Experiment 3.—A steer weighing 295 kg. was given 500 mg./kg. on each of two consecutive days. On the third day, the animal was off feed and rumen motility had essentially ceased. On the fourth day, the steer appeared perfectly normal and there were no discernible after-effects.

Experiment 4.—Another steer weighing 336 kg. was given 500 mg./kg. on each of three successive days. No toxic symptoms were observerd and the animal remained on full feed.

Experiment 5.—A steer weighing 250 kg. was given 100 mg./kg. of esteron brush killer on each of fifteen consecutive days without any outward appearance of adverse effects. The animal was killed for study forty-eight hours following the last dose. Gross examination of the internal organs revealed only slight petechial hemorrhage in the duodenum and a mild diffuse irritation in the abomasum. Histological examination of tissues from the lung, heart, spleen, adrenals, pancreas, thyroid, thymus, bladder, and lymph nodes revealed no abnormalities. Sections from the liver revealed small areas of focal hemorrhagic necrosis surrounded by areas of fatty degeneration. In the kidneys, very slight interstitial edema and congestion in the medulla and cortical medullary region were observed. Although gross examination of the gastrointestinal tract revealed mild irritation in the duodenum and abomasum, sections of these organs failed to indicate any changes of significance.

Kuron (H-1078).—Because of the current interest in derivatives of chlorinated phenoxypropionic acid, one of the more promising formulations, kuron (table 1), was included in the work with cattle. A steer weighing 250 kg. was given 100 mg./kg. of kuron on each of fifteen consecutive days. No adverse effects were noted when judged by general appearance, behavior, and weight gain. The animal was killed for examination forty-eight hours following the last dose. Gross examination of the in-

TABLE 4—Summary of Acute Oral Toxicity of Various Herbicidal Formulations (Dow)

Material	Species	Sex	Vehicle	LD50 (19/20 confidence limits) (mg./kg.)
2,4-Dow weed killer	Guinea pigs	F	Water	2,000 Range (1,000-3,000)
2,4-Dow weed killer (formula 40)	Rats	F	Water	850 Range (700-1,000)
Esteron 44	Rats	M	Olive oil	850 Range (300-1,000)
Esteron 245 (old)	Rats	F	Emulsion in water	1,000 Range (300-2,000)
Esteron 245 (new)	Rats	M	Olive oil	400 (300-1,000)
Esteron ten-ten	Rats	F	Olive oil	760 (660-860)
Brush killer 50-50	Rats	F	Emulsion in water	1,070 (700-1,650)
	Guinea pigs	F	Emulsion in water	1,160 (820-1,630)
	Mice	F	Corn oil	800 (624-1,070)
	Rabbits	F	Undiluted	1,420 Range (500-2,000)
	Chicks	M and F	Undiluted	4,000 (2,700-5,900)
Brush killer T	Rat	F	Emulsion in water	1,200 (783-1,850)
	Guinea pigs	F	Emulsion in water	1,410 (873-2,290)
	Mice	F	Olive oil	1,230 (978-1,620)
	Rabbits	M	Undiluted	849 (604-1,190)
	Chicks	M and F	Undiluted	2,000 (1,350-2,960)
Brush killer 76	Rats	F	Corn oil	730 Range (500-1,000)
Brush killer 76E	Rats	F	Corn oil	500 Range (250-1,000)
Esteron brush killer (old)	Rats	M	Emulsion in water	1,000 Range (300-3,000)
Esteron brush killer (new)	Rats	M and F	Corn oil	860 (800-930)
	Guinea pigs	M	Corn oil	1,220 (1,040-1,430)
	Guinea pigs	F	Corn oil	1,000 (1,290-1,440)
	Rabbits	M and F	Corn oil	960 (790-1,160)
	Chicks	M and F	Corn oil	2,000 Range (1,000-3,000)
	Steers		Undiluted	Greater than 1,000

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ternal organs revealed no abnormalities. Histological examination of tissues from the lung, heart, liver, spleen, pancreas, thymus, bladder, lymph nodes, rumen, reticulum, omasum, and abomasum revealed no abnormalities. Very slight interstitial edema and congestion was observed in the medulla and cortical medullary regions of the kidney, but since this has frequently been observed in untreated animals, its significance is questionable.

Discussion

Significance of Experimental Results.—Study of the data presented in table 3 indicates that the various forms of 2,4-D and 2,4,5-T all fall in the same range of toxicity for rats, mice, guinea pigs, and rabbits. The dog appears to be somewhat more susceptible to these materials than the other species studied, and chicks appear to be more tolerant. It is also apparent that MCP (2-methyl-4-chlorophenoxyacetic acid), silvex (2-[2,4,5-trichlorophenoxy] propionic acid), and their derivatives are less toxic acutely than the corresponding derivatives of 2,4-D and 2,4,5-T. The $l.d._{50}$ values for 2,4-D and 2,4,5-T and their common derivatives are in the range of 300 to 1,000 mg./kg. for the rat, mouse, guinea pig, and rabbit, whereas for MCP and silvex, the corresponding values range from 600 to 1,400 mg./kg.

The data presented in table 4 show that the acute oral toxicity of the commercial formulations, in general, tends to be proportional to that which might be expected from their content of active ingredients. Thus, it appears that the "inert ingredients" present in these formulations are less toxic than the active agents. Furthermore, the "inerts" do not appear to exert a potentiating effect upon the toxicity of the active materials. For the commercial formulations studied, the $l.d._{50}$ values for rats, mice, guinea pigs, rabbits, and cattle range from 500 to 2,000 mg./kg.; for chicks the range is from 2,000 to 4,000 mg./kg.

The results of repeated oral administrations indicate that 2,4-D and 2,4,5-T can be tolerated without adverse effects in doses only slightly smaller than those which cause toxic effects when given only once. This fact demonstrates that these materials have a low degree of chronicity.

The studies with cattle have demonstrated the similarity in susceptibility of cattle and small laboratory animals to this type of herbicidal materials. It has also been demonstrated that cattle are distinctly more tolerant of this type material than dogs.

The following conclusions with regard to cattle seem justified:

1) A daily dose of 30 mg./kg. probably can be tolerated for prolonged periods without adverse effect.

2) A daily dose of 100 mg./kg. would not be expected to cause any ill effects unless exposures were continued for a week or longer. However, if exposures were continued, some liver and kidney injury, and perhaps some gastrointestinal irritation could occur. Death or serious illness would not be anticipated.

3) A daily dose of 500 mg./kg., if continued for several days, could result in serious effects. A single dose of 500 mg./kg. would not be likely to cause serious effects.

4) A single dose of 1,000 mg./kg. may or may not cause illness. Repeated doses of this size are very likely to cause death.

Evaluation of Direct Hazard to Livestock.—In actual practice, esteron brush killer (a formulation of esters of 2,4-D and 2,4,5-T) and similar formulations are not ordinarily used on areas containing appreciable forage at a rate greater than 2 quarts per acre or 47 mg./sq. ft.

Assuming that *all* of the herbicide applied at this dosage rate is deposited on edible forage, an animal weighing 350 kg. (770 lb.) would have to consume *all* the forage on 744 sq. ft. of treated area to acquire a dose of 100 mg./kg. of the herbicidal formulation. Such a dose could be tolerated daily for a number of days without any serious effects. Based on the same assumption, it would be necessary for the animal to graze completely an area ten times as large, or 7,440 sq. ft. (0.17 acres) in order to acquire an acutely toxic dose (approximately 1,000 mg./kg.).

Since these assumptions are based upon *complete* availability to livestock of *all* the herbicide applied, an obviously impossible condition, it is apparent that there is little, if any, direct hazard to livestock or wildlife foraging areas treated with herbicides of the type described herein. This has been confirmed by the extensive use of these ma-

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terials over a number of years without any proved cases of adverse effects.

Evaluation of Indirect Hazard to Livestock.—There has been much discussion of the possibility that spraying with these herbicides might cause some plants to become toxic, toxic plants to become more toxic, and ordinarily nonpalatable plants to become palatable. Considerable investigation of these possibilities has resulted in the general conclusion that from a practical standpoint these suppositions were more fanciful than factual.

In 1950, Willard [11] of Ohio State University and Agricultural Experiment Station pointed out that the spraying of certain types of plants with 2,4-D increased their nitrate content to hazardous levels.

The leaves of sugar beets accidentally sprayed with 2,4-D were reported to have caused the poisoning of some livestock. However, since 2,4-D is never knowingly used on sugar beets, this does not appear to present a serious practical hazard. Other weeds such as lambsquarter, pigweed, and smartweed have also been reported as containing increased quantities of nitrate after treatment with 2,4-D. Since livestock do not ordinarily eat these species, it would seem that the hazard presented by the accumulation of nitrate in these plants is slight. The lack of reported cases of nitrate poisoning would seem to substantiate this supposition.

Wild cherry is recognized as a hazardous plant to have in grazing areas. Couch [9] has suggested that the wilting of wild cherry leaves results in the hydrolysis of cyanogenic glucosides to form free hydrocyanic acid (HCN) and, therefore, that cut wild cherry is much more hazardous to livestock than the growing plants. By analogy, it has been postulated that wild cherry leaves wilted as a result of treatment with 2,4-D and 2,4,5-T herbicides likewise might be more toxic than growing leaves. Barrons and Lynn [9] and Grigsby and Ball,[10] working independently, demonstrated clearly that the HCN content of wild cherry leaves sprayed with esteron brush killer was actually lower than the content of unsprayed leaves.

Dr. Willard also has called to attention the possibility that treatment with 2,4-D type herbicides might make certain naturally poisonous plants more palatable or even attractive to livestock. This is a possibility but, so far, it has not been established as a fact.

In evaluating the hazard associated with the forementioned possibilities, Dr. Willard in 1951 [12] stated as follows: "In the first place, literally dozens of carefully controlled feeding experiments, both with 2,4-D and 2,4,5-T directly and with vegetation sprayed with them, have uniformly and without exception failed to show any poisonous effects on any kind of livestock.

"In the second place, literally millions of acres of pastures with livestock on them have been sprayed in the last six or seven years without injury to livestock. I am sure there is no experimenter here who has not used 2,4-D in occupied pastures. In our state, one company over a period of four years has sprayed 60,000 miles of roadside in twenty-six counties. During that time, he has had only five reports of suspected poisonings, none of them substantiated, 2 of swine, 2 of cattle, 1 of sheep. A company in Pennsylvania, which has sprayed 10,000 acres of right-of-ways per year, reports just 4 cases, only 1 of which was not completely cleared up as due to other causes, in five years of work."

In 1953, Dr. S. N. Fertig [13] of Cornell University summarized the situation regarding poisoning as follows, "There are no known cases of actual herbicidal poisoning from field application of presently used herbicides marketed as nonpoisonous.* There is no sound toxicological evidence available of the presently publicized nitrate-phenol and nitrite poisoning as a common cause of death. True, nitrate and nitrite poisoning has occurred in cattle, sheep, and swine from eating plants grown on high nitrate soils or from other feed sources. None of these, however, have been definitely traced or shown to be caused by herbicidal treatment. All the alleged cases of herbicidal poisoning of livestock and wildlife that have been definitely diagnosed have been caused by one of the following: (1) lead, (2) arsenic, (3) hardware disease, (4) poisonous plants, (5) old age, (6) parasites, (7) drowning, (8) poor marksmanship, (9) contaminated food, and (10) injection or oral dosage of some medicine or drug. In all cases that have been carefully surveyed, even though the herbi-

* Dr. Fertig's classification of herbicides marketed as non-poisonous includes such materials as those discussed in this paper.

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cide has been associated with the trouble, it has in no case been directly or indirectly related to the deaths reported."

SUMMARY AND CONCLUSIONS

1) The acute oral $l.d._{50}$ values for 2,4-D, 2,4,5-T, and their various derivatives commonly used in herbicidal preparations fall in the range of 300 to 1,000 mg./kg. for rats, mice, guinea pigs, and rabbits. Generally, dogs are more susceptible and chicks are more tolerant of this type of material. On a weight basis, the toxicity to cattle appears to be quite similar to the toxicity to ordinary laboratory animals.

2) The acute oral $l.d._{50}$ values for commercial formulations of 2,4-D and 2,4,5-T type materials are approximately proportional to their content of active ingredient. The "inert ingredients" do not appear to contribute materially to the oral toxicity of the formulations.

3) Data available indicate that the 2,4-D and 2,4,5-T type herbicides have a low chronicity.

4) MCP (2-methyl-4-chlorophenoxyacetic acid) and silvex (2-[2,4,5-trichlorophenoxy] propionic acid) and their herbicidal derivatives appear to be slightly less toxic than the corresponding 2,4-D and 2,4,5-T type materials.

5) The hazard to livestock and wildlife associated with the use as recommended of herbicides containing 2,4-D, 2,4,5-T, MCP, and silvex is negligible. It should be recognized, however, that toxic amounts of these materials can be obtained if animals have access to spray tanks or other containers of the materials.

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DOWN to EARTH

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Applying 2,4,5-T in Diesel oil to hardwood with Cornell tool. The device is heavy, much like a spud, and permits the injection of the chemical in cuts in the basal area.

KILLING SMALL UNDESIRABLE HARDWOODS *by Use of the CORNELL TOOL*

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Results of a small scale study, established in July and August, 1952, on the W. Goodrich Jones State Forest near Conroe, Texas, indicate that 2,4,5-T applied with a Cornell tree poisoning tool may be an effective method of releasing young pines from competing undesirable hardwoods.

Plots, one-tenth acre in size, were set up to compare the effectiveness of 2,4,5-T ester applied as a basal spray and with a Cornell tool. The propylene glycol butyl ether ester was used in each case. A 2½ per cent solution of 2,4,5-T, by volume, in diesel oil (10 lb. acid equivalent per 100 gallons of spray), was applied by the basal spray method to all hardwoods on two plots in July and August, 1952. Solutions of 10 and 20 per cent 2,4,5-T in Diesel oil (10 and 20 percent 2,4,5-T, 40 and 80 lb. acid equivalent per 100 gallons of spray) were applied with the Cornell tool to trees in two plots in July. In August, one additional plot was treated with a 10 per cent solution in Diesel oil and one plot was treated with a 10 per cent emulsion in water, both applied with the Cornell tool.

As both application methods are primarily useful in the treatment of small trees, the plots were located in a stand dominated by sapling size hardwoods. However, the few larger hardwoods found on the plots were treated by the various methods being tested. Species predominating were redgum, blackgum and southern red oak. Smaller numbers of post, white and water oaks, yaupon, holly, sassafras, tree huckleberry, red mulberry and red haw were also found on the plots.

With basal spray applications, the bark of treated trees was wet to the point of run-off from the root collar to approximately one foot above the ground. Cornell tool cuts were spaced approximately one inch apart around the tree base. Table I indicates average diameter of treated trees and the average volume of each solution used per tree.

TABLE I

VOLUME OF CHEMICAL SOLUTIONS AND AVERAGE SIZE OF TREES TREATED BY BASAL SPRAY AND CORNELL TOOL METHODS

Method of Application	*Concentration (by volume) Per Cent*	*Diluent*	*Average volume used per tree* m.l.*	*Average diameter of treated trees inches*
Basal spray	2½	Diesel oil	200	3.7
Cornell tool	10	water	8	2.6
Cornell tool	10	Diesel oil	11	3.2
Cornell tool	20	Diesel oil	11	3.3

* Average determined from the total amount used per plot.

In September, 1953, a little over one year after treatments were applied, 100 per cent of the hardwoods 10 inches in diameter and smaller treated with 20 per cent 2,4,5-T by the Cornell tool method were dead. Of these, only 1.6 per cent have resprouted. The 10 per cent 2,4,5-T in Diesel oil treatment was also effective. A total of 88 per cent of hardwoods under 10 inches in diameter were dead.

Hardwood mortality was less and sprouting more prolific in the plot where the 10 per cent 2,4,5-T water emulsion was applied with the Cornell tool.

Trees treated with 2,4,5-T by the basal spray method usually die more slowly than when fresh wounds are treated. On the basal spray plots, 53 per cent of the hardwoods under ten inches in diameter were dead one year after treatment. By the start of the second spring after treatment, 63 per cent were dead. Many of the trees still surviving at that time were in a weakened condition and had been drastically reduced in living crown.

The results of these treatments are summarized by species groups in Table II. As only two larger trees have died as a result of any of these treatments, only those less than ten inches in diameter have been included in these data.

TABLE II—PERCENTAGE OF DEAD HARDWOODS 10 INCHES D. B. H. AND UNDER TWO YEARS AFTER TREATMENT WITH BASAL SPRAY CORNELL TOOL

SPECIES	BASAL SPRAY TREATMENTS			CORNELL TOOL TREATMENTS								
	2½%, 2,4,5-T in Diesel Oil			*10% 2,4,5-T in Water*			*10% 2,4,5-T in Diesel Oil*			*20% 2,4,5-T in Diesel Oil*		
	Number of trees treated	*Per cent of trees dead*	*Per cent of trees sprouting*	*Number of trees treated*	*Per cent of trees dead*	*Per cent of trees sprouting*	*Number of trees treated*	*Per cent of trees dead*	*Per cent of trees sprouting*	*Number of trees treated*	*Per cent of trees dead*	*Per cent of trees sprouting*
Redgum	70	63	0	49	49	4	49	88	0	47	100	0
Blackgum	17	41	0	15	33	20	20	90	0	10	100	10
Oaks[1]	14	57	12	57	83	23	40	88	3	5	100	0
Yaupon & Holly	6	100	0	5	60	0	7	100	0	1	100	0
Miscellaneous[2]	11	90	0	3	100	0	4	75	0	1	100	0
All species	117	63	2	129	61	17	120	88	1	64	100	2

[1] Includes mostly southern red oak with a few post, white and water oaks.
[2] Includes sassafras, wax myrtle, tree huckleberry, sumac, mulberry and red haw.

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Determination of 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Grain and Seed

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The 2,4-D acid is extracted from a dry sample of meal with an acidified ether-chloroform solvent. By further treatment, including a chromatographic separation, interfering materials are removed. The color produced by the reaction of 2,4-D acid with chromotropic acid in concentrated sulfuric acid is measured on a suitable spectrophotometer. As little as 5 γ of 2,4-D acid in a 200-gram sample can be detected.

THE USE OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D acid) as a weed killer on a variety of crops has made it desirable to develop an analytical method capable of detecting minute quantities of 2,4-D acid which may be picked up by the plant. The possibility of translocation of the 2,4-D acid must also be determined.

The method of Marquardt and Luce (*4*) was not adaptable directly to this problem, and the procedure of Gordon and Beroza (*3*) did not offer a means of separation from the substrata or indicate the possibility of detecting only trace ntities.

A method of extraction and separation of the 2,4-D acid was developed and the colorimetric method of Freed (*2*) further refined to give the accuracy demanded for this problem.

Reagents

Chloroform, technical grade. Ether, U.S.P. grade. Acetic acid, glacial. Sodium hydroxide, approximately 5*N*. Hydrochloric acid, concentrated. Sodium hydroxide, approximately 0.5*N*.

Phosphotungstic acid. Dissolve 40 grams of phosphotungstic acid (approximately $P_2O_5.24WO_3.25H_2O$) in water and dilute the solution to 100 ml.

Hyflo Super-Cel, a Celite product, diatomaceous silica, made by Johns-Manville.

Absolute methanol in U.S.P. chloroform, 3.0 and 7.0% by volume (see section on chromatographic separation of 2,4-D acid).

Buffered extraction solution, pH 6.85. Dissolve 19.0 grams of dibasic sodium phosphate, $Na_2HPO_4.7H_2O$, and 10.0 grams of monobasic sodium phosphate, $NaH_2PO_4.H_2O$, in water and dilute to exactly 1.0 liter.

Chloroform, analytical reagent grade.

Chromotropic acid. Dissolve 0.10 gram of the sodium salt of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) in 100 ml. of concentrated sulfuric acid (95.5%).

Sulfuric acid–stannous chloride solution, dilute. Mix 200 ml. of concentrated sulfuric acid with 800 ml. of water and cool the dilute acid to room temperature; then dissolve 50 grams of stannous chloride, $SnCl_2.2H_2O$, in the acid.

2,4-Dichlorophenoxyacetic acid, standard solution. Dissolve 0.500 gram of 2,4-D acid in chloroform and dilute to 500 ml. Dilute a 10.0-ml. aliquot to 100 ml. with chloroform. Dilute a 10.0-ml. aliquot of this second solution to 100 ml. with chloroform. The final solution contains 0.01 mg. of 2,4-D acid per ml.

Apparatus

The tube for the chromatographic column is a glass tube, 17 mm. in inside diameter and 50 cm. long, one end of which is constricted and attached to a smaller glass tube, 5 mm. in inside diameter and 10 cm. long.

A suitable apparatus for regulating air pressure and an interval timer are also required.

A Coleman spectrophotometer, Model 11, equipped with a PC-4 filter and 5-cm. cuvettes, was used in this investigation. Any photometer measuring light transmittance at 565 mμ should be suitable.

Procedure

Grind the grain or seed to a fine meal to pass through a No. 10 sieve. In this work a No. 1 Wiley mill was used.

Place a 200-gram sample of the meal in a 2-liter glass-stoppered bottle, and add 500 ml. of technical grade chloroform, 500 ml. of ether, and 10 ml. of acetic acid. Stopper the bottle, fastening the stopper with Okonite tape. Place the bottle and contents in a shaking machine for 1 hour.

Use a 2-liter filtering flask, a Büchner funnel 16 cm. in diameter, and Whatman's No. 1 or similar grade filter paper. Rinse the bottle by adding 50 ml. of technical grade chloroform and 50 ml. of ether and wash the insoluble material with the rinsing solution.

Discard the insoluble material. Using a 2-liter separatory funnel, extract the 2,4-D acid from the filtrate once with a solution of 200 ml. of water and 50 ml. of 5*N* sodium hydroxide and twice with a solution of 200 ml. of water and 10 ml. of 5*N* sodium hydroxide. Shake well each time to ensure complete extractions. Discard the organic layer and wash the combined aqueous extracts three times with 100-ml. portions of technical grade chloroform.

Acidify the aqueous solution with 50 ml. of concentrated hydrochloric acid. Extract the 2,4-D acid from the solution with 125 ml. of ether. Repeat the extraction with 100 ml. of ether and combine the extracts. Discard the aqueous solution.

Filter the ether solution, using Whatman's No. 1 or equivalent grade filter paper, into a 250-ml. separatory funnel. Extract the 2,4-D acid from the filtered solution once with 50 ml. of 0.5*N* sodium hydroxide and twice with 25-ml. portions of 0.5*N* sodium hydroxide. Combine the caustic extracts.

Pour the caustic solution into the 250-ml. separatory funnel and wash it three times with 10-ml. portions of chloroform, discarding the washings. Acidify the solution with 10 ml. of concentrated hydrochloric acid and add 5 ml. of phosphotungstic acid solution. Extract the 2,4-D acid with three 10-ml. portions of chloroform and combine the extracts.

Prepare the column for the chromatographic separation by placing a plug of glass wool in the bottom of the tube at the constriction. Add Hyflo Super-Cel and settle it in the tube by bouncing the tube gently on a large rubber stopper. Continue adding more Super-Cel and bouncing the tube gently until the Super-Cel is packed in the tube for a distance of 20 cm.

Filter the chloroform solution into the tube, using No. 1 Whatman's or equivalent grade filter paper. Apply air pressure at the top of the tube to push the solution into the Super-Cel.

Using proper air pressure, percolate 150 ml. of the 3.0% methanol in chloroform solution through the column at the rate of 150 ml. per hour, followed by 35

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mended exclusively. Operators should study their particular conditions in the light of available information to determine the most profitable methods to follow.

Most chemical research work on woody plant control on range or pasture land should be guided by these fundamental objectives: (1) development of effective treatment, (2) cost and possible net return and (3) range management practices necessary for continued benefits. Some federal and state research men who have contributed materially to chemical brush control in the South are: C. E. Fisher at Spur, Texas on mesquite; Dave Savage and Pat McIlvain at Woodward, Oklahoma on sagebrush and Shinnery oak; Fred Peevy and John Cassady on Louisiana oaks; L. E. Chaiken on hardwoods in North Carolina; Harry Elwell on oak brush at Guthrie, Oklahoma; and R. A. Darrow and Wayne McCully on post and blackjack oaks, prickly pear, whitebrush and huisache in Texas. This work has guided further research and demonstrations in the South.

Chemicals used in brush control work may be selective such as 2,4-D, 2,4,5-T and silvex or nonselective such as ammonium sulfamate, arsenic and kerosene. Fundamentals of the ultimate in a woody plant control chemical are: (1) nonpoisonous to man or livestock; (2) effective under a wide range of conditions; (3) cheap; (4) easy to apply and to handle; (5) not damaging to grass or cultivated crops. Present chemicals meet some of these requirements but not all of them.

Chemicals may be applied by broadcast methods or by individual tree or plant treatment. Tractor or jeep drawn power spray equipment can be used on low growing brush or following mechanical control work. Aerial application provides economical and rapid coverage of large brush areas. Such broadcast methods have certain limitations. At the present time. most chemicals applied when the brush is in full foliage, control only certain kinds of brush and must be used very carefully in susceptible crop areas.

Sprays may be applied to individual plants with hand operated or cattle sprayers. Such equipment is suitable for foliage treatment of scattered individual seedlings or small brush and for frill, basal and stump treatment.

Extensive brush control research conducted at the U. S. Southern Great Plains Field Station at Woodward, Oklahoma shows that three-fourths of the sand sagebrush can be killed with one proper aerial application of 2,4-D. Forage and beef production is increased from 50 to 75 percent if grazing is light or summer deferment is practiced following treatment. The cost of sagebrush spraying on experimental areas has been repaid completely the first year following treatment.

More than one million acres of mesquite have been aerially sprayed in Texas during the past 3 years with ½-¾ lb. of 2,4,5-T (as low-volatile ester) per acre in diesel oil at a cost of $3.00 to $3.50 per acre. Top kills will average around 90 percent and root kills 40 percent. Mesquite is the biggest one species problem in Texas with a dense stand on 35 million acres and scattered stands on another 15 million acres. It is a prolific sprouter from the bud zone. Stage of growth, moisture condition, size of trees and management following treatment largely determine the benefits derived from spraying. Grass growth and beef production have been materially increased on all areas sprayed and the writer has yet to visit an area on which mesquite spraying has not paid for itself.

Aerial control of post oak and blackjack oak has been tried on a limited scale. It appears practical and economical (Figure 1). Two pounds of 2,4,5-T acid equivalent per acre (as low volatile ester) should be applied after plants have reached full leaf in the spring. The cost will be about $8.00 per acre but these oak areas have more favorable rainfall and will produce more forage than many mesquite areas. It should be emphasized that for both mesquite and oak, control rather than eradication is the result. Additional sprayings will be necesary to control regrowth. Can all kinds of upland and bottomland hardwoods be aerially controlled economically? There is a need for more research on this problem. Aerial application is the cheapest method known with the limitation that acreages must be large and no susceptible crops are close by.

Possibly hardwoods may be controlled by aerial application of 2,4,5-T, silvex, or MCP in timber areas. Earlier research indicated chemicals would kill pines but later work makes researchers wonder. These possibilities should be fully explored.

Chemicals may be applied to the foliage of individual trees, to the cut-off stump, trunk base of standing trees or in cups or frills made in the bark. 2,4,5-T ester and ammonium sulfamate are the most widely used and accepted chemicals. In Texas and Oklahoma, 2,4,5-T has generally given cheaper and more effective results with less labor.

Stump treatment using 2,4,5-T ester diluted in diesel oil or kerosene gives good control of sprouting for most species (Figure 2). Trunk base treatment using 2,4,5-T in oil gives good results on trees under six inches in diameter but some species require twice the concentration of chemical as others (Figure 3). Ammonium sulfamate crystals in cups spaced not over six inches apart and close to the ground or applied in water solution to frills usually controls sprouting on oaks. A solution of 2,4,5-T in oil applied in single overlapping axe cuts shows much promise of cheap, effective control of all kinds of trees.

The value of any program is dependent upon its acceptance by the people. Extension woody plant control in Texas has been presented through county agent training meetings, method demonstrations in counties, newspaper, magazine and radio stories and TV demonstrations. In 1952, working with the county agents, the writer conducted brush control meetings or demonstrations in 46 different counties. In 25 counties, 33 field demonstrations were set up using 2,4,5-T and ammonium sulfamate on stumps, trunks and in frills. These demonstrations were permanently marked with the kinds of chemical used and method of treatment. Last year, 40 brush control meetings and 30 field demonstrations in 24 different counties were held. For the past two years, this gives a total of 86 brush control meetings, 63 field demonstrations in 49 different counties and 150 hardwoods demonstrations conducted by county agricultural agents and this specialist. As a result of an evaluation of these field demonstrations together with experiment station results, a circular, "More Grass from Controlling Hardwoods with Chemicals" C-330, has been prepared for the guidance of Texas farmers and ranchmen and is available from the Texas Agricultural Extension Service.

A Challenge

Whereas brush control is the biggest problem facing the farmer, ranchman and timber producer in many southern areas, it also presents a very great opportunity. In business 3 or 4 percent interest is considered a fair investment while the application of present woody plant control information can result in 20 to 50 per cent increase in desirable production and 100 per cent gain is not uncommon when coupled with proper range, pasture and timber management.

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Summary of Toxicological Information on 2,4-D and 2,4,5-T Type Herbicides and an Evaluation of the Hazards to Livestock Associated with Their Use

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THE USE OF herbicides for the control of undesirable vegetation has been practiced for many years. Some of the older materials commonly used are chlorates, arsenicals, compounds of boron, certain oils, and even table salt. While many of these materials are still being used for this purpose, research over the past decade has revealed a family of new herbicidal materials. They are the chlorinated phenoxyacetic acids and closely associated compounds of which 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) are prototypes.

Early in the development of these new herbicides, studies were undertaken by The Dow Chemical Company to determine their toxicity to warm-blooded animals. It is the purpose of this paper to make the acquired toxicological information available to others.

LITERATURE REVIEW

Bucher[1] in 1946 was among the first to report the results of experiments with small animals using 2,4-D. Temporary myotonia lasting from eight to twenty-four hours or more following a single injection of 150 to 250 mg./kg. was observed in mice, rats, rabbits, and dogs. Repeated injections of smaller amounts, 50 to 100 mg./kg./day to mice for ninety days, failed to elicit either a characteristic chronic syndrome or a striking histological picture. Mice undergoing this treatment became pregnant and bore apparently normal litters. Repeated injections of 2,4-D did not alter the rate of growth of two transplantable mouse sarcomas.

In 1947, Hill and Carlisle[2] published the results of toxicological studies involving various preparations of 2,4-D. In acute oral studies, they found the $L.d._{50}$ for mice to be 375 mg./kg.; for rats, 666 mg./kg.; for rabbits, 800 mg./kg.; and for guinea pigs, 1,000 mg./kg. The largest dose administered to monkeys without serious after-effects was 214 mg./kg.; 428 mg./kg. caused nausea, vomiting, lethargy, muscle incoordination, and head drop. These workers observed that all species reacted similarly and that there was no significant difference in potency between crude and purified preparations, or between the sodium or ammonium salts. Deaths from large doses were believed due to ventricular fibrillation; if death was delayed, myotonia, stiffness of extremities, ataxia, paralysis, and coma were observed.

In subacute studies, the same investigators observed intoxication in dogs after six daily intravenous injections of 25 Gm./kg.; rats were fed a diet containing 1,000 parts per million (p.p.m.) of 2,4-D for a month without harmful effects; guinea pigs tolerated ten doses of 100 mg./kg. over a twelve-day period; and the inhalation of the sodium salt as a dust failed to cause systemic effects in guinea pigs. The main evidences of chronic intoxication observed in rats were visceral congestion and edematous kidneys with degenerative changes in the tubules; only dogs exhibited hepatic damage with central degeneration and congestion.

In 1948, Bjorn and Northen[3] reported the results of studies conducted on chicks with an alkanolamine salt of 2,4-D. The acute oral lethal range was found to be 380 to 765 mg./kg. When given repeatedly (12 doses in 28 days), 28 mg./kg./dose was without effect, while 280 mg./kg./dose caused depression of growth. These authors express no concern about the likelihood of toxic effects in chickens under ordinary conditions of use and point out that at a spraying rate of 1 lb. of 2,4-D per acre (a normal rate of application) a chicken weighing 1 kg. would have to consume all the 2,4-D applied on 72 sq. ft. within a day or two to obtain a lethal dose.

In 1953, Drill and Hiratzka[4] reported the results of acute and chronic oral toxicity studies on 2,4-D and 2,4,5-T with dogs. These men found that the acute oral $L.d._{50}$ values were about 100 mg./kg. for each of the materials. At this dosage level, 2,4-D produced definite myotonia accompanied by anorexia and weight loss while 2,4,5-T produced only signs of mild spasticity. Both 2,4-D and 2,4,5-T were fed five times a week for ninety days at dosage levels of 2, 5, and 10 mg./kg. without adverse effects. At a dosage level of 20 mg./kg., both materials caused serious effects; 3 of 4 dogs on the 2,4-D died while all of the 4 dogs on 2,4,5-T died. The animals receiving the 2,4-D exhibited stiffness of the hind legs, difficulty in swallowing, bleeding of the gums, necrotic changes in the buccal mucosa, and mild liver and kidney changes. A significant decrease in the number of blood lymphocytes was observed terminally in 3 of the 4 animals. The toxic symptoms noted in the animals

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ml. of 7.0% methanol in chloroform. Discard the effluent.

Then percolate 150 ml. of 7.0% methanol in chloroform through the column at the same rate, collecting the effluent in a 250-ml. beaker.

Pour the effluent into a 250-ml. separatory funnel. Extract the 2,4-D acid from the solution with three 25-ml. portions of buffered extraction solution and combine the extracts.

Return the extract solution to the separatory funnel and wash it three times with 10-ml. portions of analytical reagent grade chloroform. Discard the washings.

Acidify the solution with 2 ml. of concentrated hydrochloric acid and extract the 2,4-D acid with three 10-ml. portions of analytical reagent grade chloroform. Combine the extracts in a 30-ml. beaker.

Decant the chloroform solution from the globules of water adhering to the beaker into another clean, dry 30-ml. beaker. Gently evaporate the chloroform by use of a steam bath (test tube clamps may be used to hold the beakers deep in the steam bath to facilitate the evaporation). Evaporate just to dryness and remove the beaker immediately. Cool.

Add 5.0 ml. of chromotropic acid reagent to the residue in the beaker and swirl to make a homogeneous solution. Place the beaker and contents in an oven set at 150° ± 2° C. for exactly 10.0 minutes (use interval timer). Cool the solution to room temperature (wine-purple colored if 2,4-D is present).

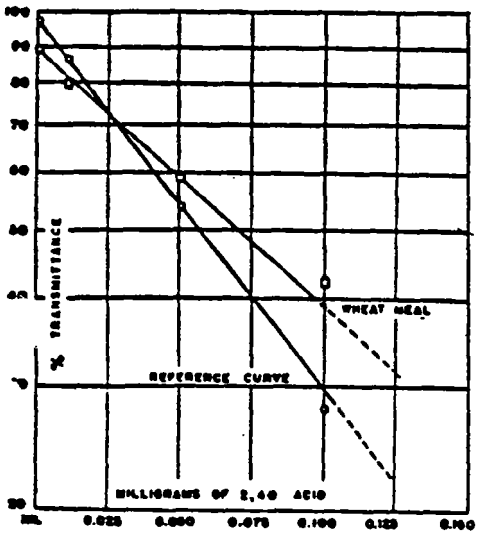
Pour the solution with stirring into about 30 ml. of sulfuric acid–stannous chloride solution, rinsing the beaker with a few milliliters of the solution. Start timing with the interval timer and cool the solution to approximately room temperature, using a cold water bath.

After about 5 minutes, pour the solution into a 50-ml. volumetric flask and make to volume with sulfuric acid–stannous chloride solution. Mix well and filter through a Whatman's No. 42 or equivalent grade of filter paper.

After 30 to 45 minutes, determine the per cent transmittancy of the filtrate at 565 mμ, using 5-cm. cuvettes with water as the reference liquid set at 100%.

Read the milligrams of 2,4-D acid contained by the sample from a graph made from the data obtained by analysis of untreated meal to which known quantities of 2,4-D acid had been added.

Figure 1. Recovery of 2,4-D acid from wheat meal



Calculation

Calculate the parts per million of 2,4-D acid present in the sample as follows:

$$\frac{\text{Mg. of 2,4-D acid} \times 1000}{\text{grams of sample}} = \text{p.p.m. of 2,4-D acid}$$

Preparation of Graph

Add 0.0, 1.0, 5.0, and 10.0 ml. of the standard solution containing 0.01 mg. of 2,4-D acid per ml. to 200-gram samples of untreated meal, and determine 2,4-D acid according to the procedure. Using the data obtained, construct a suitable graph.

Data obtained by the authors on wheat meal are shown in Table I.

Reference data on known amounts of 2,4-D acid were obtained by pipetting 0, 1.0, 5.0, and 10.0 ml. of the standard solution containing 0.01 mg. of 2,4-D acid per ml. into four 50-ml. beakers and gently evaporating the chloroform by use of a steam bath. Then 5.0 ml. of chromotropic acid reagent were added and the color was developed according to the procedure. Results are given in Table II.

The data from Tables I and II are shown graphically in Figure 1. Comparison of the two sets of data shows that the recovery of 2,4-D acid, although not complete, is adequate to detect and estimate trace quantities of the acid in wheat meal. The over-all loss is corrected in the preparation of the graph.

Graphs made for meals of barley, flax, dried peas, and oats were similar to the one prepared for wheat meal. For oats, it was found expedient to use 100-gram samples.

The analytical data found on the samples of grain and seed listed above correlate very well with the information available in each case.

Chromatographic Separation of 2,4-D Acid

The developing solution and the eluting solution used with the Hyflo Super-Cel for the chromatographic separation of the 2,4-D acid should be carefully standardized in order to make a proper separation.

The adsorptive strength of Hyflo Super-Cel was found to vary somewhat from batch to batch. Therefore, a uniform batch of Super-Cel, in adequate supply for standardization of the chromatographic separation and for use in many subsequent separations, should be set aside.

Table I. Transmittancy Values for 2,4-D Acid in Wheat Meal

2,4-D Acid, Mg.	*% Transmittancy*
Nil (blank)	89.0
0.01	79.8
0.05	58.8
0.10	42.0

Table II. Transmittancy Values for 2,4-D Acid

2,4-D Acid, Mg.	*% Transmittancy*
Nil (blank)	97.1
0.01	86.2
0.05	53.7
0.10	27.8

The presence of water was found to affect the elution strength of the solutions used in the chromatographic separation. Since the water content in technical methanol may vary from drum to drum, several gallons of methanol from a selected drum should be stored and used for standardizing the solutions and for the duplication of these solutions thereafter. In this investigation, methanol containing 0.03% water was used.

It is probably not necessary to store chloroform, as variation in quality from various lots appeared to have no significant effect on the elution strengths of the solutions.

Prepare the column for the chromatographic separation by packing the tube with Super-Cel for a distance of 20 cm. Pour about 30 ml. of chloroform containing 0.10 mg. of 2,4-D acid into the tube. Apply air pressure at the top of the tube to force the solution into the Super-Cel.

Percolate 150 ml. of 3.0% methanol in chloroform solution through the column at the rate of 150 ml. per hour, followed by 25 ml. of 7.0% chloroform in methanol. Discard the effluent. Then pass 50 ml. of 7.0% methanol in chloroform through the column at the same rate and save the liquid in a 100-ml. beaker. Repeat this three times with the similar portions of the eluting solution.

Test for 2,4-D acid in each beaker as follows: Evaporate the liquid by use of a steam bath, add 10 ml. of chromotropic acid reagent, and swirl to make a uniform solution with any residue. Place the beaker and contents in an oven set at 150° ± 2° C. for 10 minutes. Then examine the solutions for the wine-purple color which indicates the presence of 2,4-D acid.

For a good chromatographic separation of the 2,4-D acid, results should be:

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1. At most, a faint wine-purple color in the first beaker, indicating a trace of 2,4-D acid.

2. A deep wine-purple color in the second beaker, indicating most of the original 0.10 mg. of 2,4-D acid.

3. A pale wine-purple color in the third aker, indicating only a small portion of the original 0.10 mg. of 2,4-D acid.

4. No wine-purple color noticeable in the fourth beaker, indicating the absence of 2,4-D acid.

The fourth 50-ml. portion of 7.0% methanol in chloroform is not run through the tube during a regular analysis. It is done here for testing the chromatographic separation to make sure that all of the 2,4-D acid is eluted with the first 150 ml. of 7.0% methanol in chloroform.

If the results do not show a good separation of the 2,4-D acid, the amount of methanol in the two solutions of chloroform should be adjusted.

Discussion

Phosphotungstic acid is used to separate proteins when the 2,4-D acid is extracted with chloroform from the acidified aqueous solution.

The buffered extraction solution separates the 2,4-D acid from some of the small amount of acidic material remaining with the 2,4-D acid after the chromatographic separation.

After the final separation of the 2,4-D acid, a very small amount of acidic material from the grain sample may be present, which will give an amber color when heated with chromotropic acid reagent. The stannous chloride in the dilute sulfuric acid bleaches the amber color, but under the conditions of the experiment it does not bleach the wine-purple color. Though the solution from an untreated grain sample obtained by analysis is not water-white, the per cent transmittancy is high and constant.

The color reaction of 2,4-D acid with chromotropic acid is not quite specific. Formaldehyde will produce the same wine-purple color (*1*). According to Freed (*2*), phenoxyacetic acid and its derivatives will give the same color, perhaps because formaldehyde is a decomposition product.

The procedure can probably be used for phenoxyacetic acid and many of its derivatives besides 2,4-D acid, although the solutions of methanol in chloroform may have to be modified to make a proper chromatographic separation of the desired compound. The procedure as given has been used to determine 4-chloro-o-toloxyacetic acid (MCP acid).

Although this procedure was developed principally for the determination of 2,4-D acid in grain and seed, it can probably be used for other agricultural products. Fresh vegetables such as peas should be dried by suitable means and the analysis made on a meal of the dried sample.

It is possible to detect less than 0.05 p.p.m. of 2,4-D acid in samples by this analytical procedure. Interferences by other compounds were not experienced as, under the conditions of the procedure, the color test is specific for aryloxyacetic acids.

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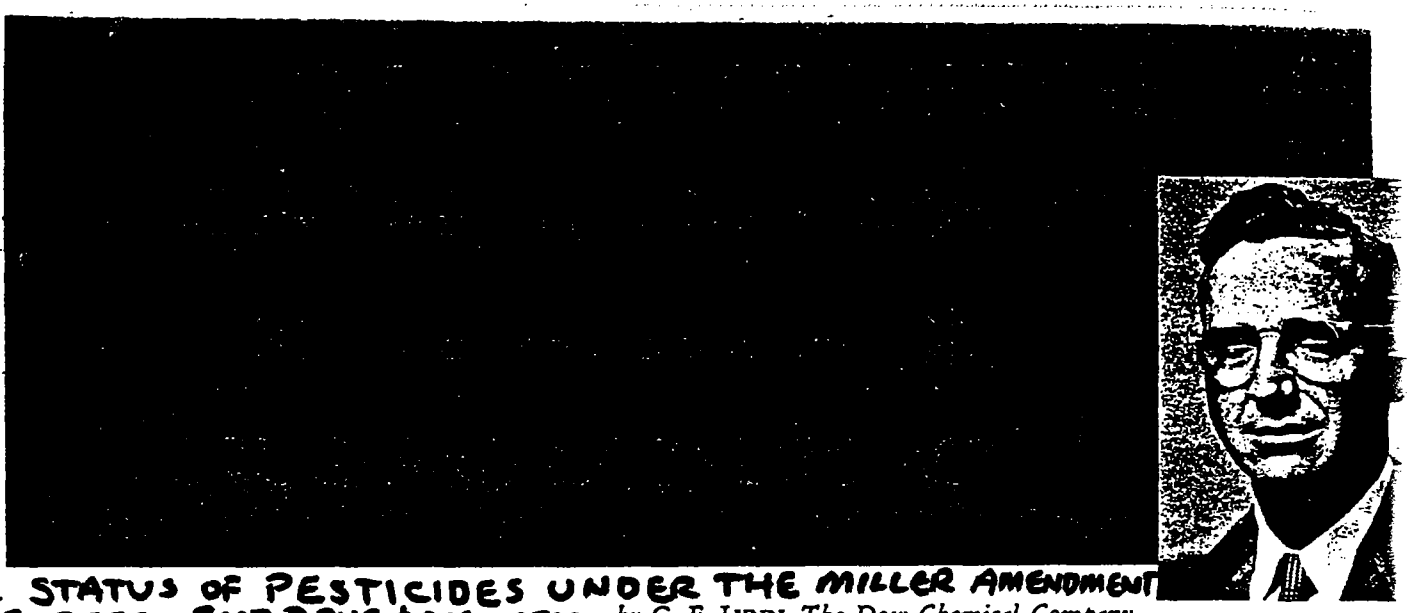
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"THE STATUS OF PESTICIDES UNDER THE MILLER AMENDMENT TO THE FEDERAL FOOD, DRUG & COSMETIC ACT"

by G. E. LYNN, *The Dow Chemical Company*

Although representatives of industry and Government have tried to clarify the status of pesticide chemicals under the Miller Amendment (Public Law 518, 83rd Congress) to the Federal Food, Drug and Cosmetic Act, there are still some misunderstandings regarding recommendations that can be made for the coming season. Mr. L. S. Hitchner, Executive Secretary of the National Agricultural Chemicals Association, speaking on the subject at The Conference on Problems Involved in the Study, Evaluation and Application of Pesticides with Special Reference to Safety of Use, Washington, D. C., October 19, 1955, said in part:

"Under present Federal legislation, industry is required to supply two basic types of data before sale in interstate commerce.

1. To the United States Department of Agriculture, information and evidence showing the effectiveness of the material and of particular interest at this time the pesticide residue remaining on a crop when used in accordance with label directions. This information, in due course, reaches, where a residue remains, the Food and Drug Administration.

2. To the Food and Drug Administration, the toxicity data required by them in order to determine a tolerance, if one is required. In establishing a tolerance the amount of residue remaining is considered.

"The residue data is obtained from work done by companies in their own laboratories; from field operations; from the work of Land Grant Colleges, both on cooperative projects and independently; from private research institutions and from expert research organizations doing work in toxicology and medicine.

"The result of this program means that adequate information on residues is deposited by the company to reasonably assure, that when used as directed, residues, if any, will be within the tolerance determined by the Food and Drug Administration. Under this program the grower is reasonably assured that when used as directed there is no excess residue and the public likewise is assured there is no undue hazard to the public health.

"As a practical matter, growers and others will have to depend primarily on the label to assure there is no excess residue. All instructions on the label, including directions for use, timing of applications and other conditions must be explicitly followed."

Mr. Hitchner also pointed out that there were, "six categories in which the residue tolerance problem can fall at the present time and on which there seems to be considerable confusion.

1. Some products are exempt from the provisions of the Law because they are not considered poisonous or deleterious. Example: Sulphur.

2. Products which have no tolerance established. This category would include materials which leave no residue and, therefore, require no tolerance. Example: 2,4-D on small grains.

3. An exemption from a tolerance. Food and Drug may exempt materials from a tolerance because of their safe characteristics. Examples: Pyrethrum and Copper.

4. A product may have a tolerance established in so many parts per million. Example: DDT, which has a seven parts per million tolerance.

5. A zero tolerance. A zero tolerance would apply when a material can be used but only under such conditions as result in no residue when the crop is marketed. Example: Mercury. A zero tolerance is often confused with "no tolerance."

6. Certain provisions of the Act may be extended until July 22, 1956.

"Those interested in finding out where a product stands in the six categories listed above should write to the manufacturer."

* * * * * * *

STATUS OF DOW AGRICULTURAL CHEMICALS

All current labels for Dow herbicides, insecticides, fumigants and fungicides have been cleared by the Pesticide Regulation Section of the United States Department of Agriculture. In those cases in the following text where extensions are indicated it is probable that tolerances or exemptions will be established before the law becomes fully effective on July 22, 1956, and that current label directions will continue to be in effect.

HERBICIDES

1. Baron (2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate). Not presently used on food crops, therefore, no tolerance is necessary.

2. Brush Killer T, Brush Killer 50-50, Esteron Brush Killer, Esteron 245, and Kuron (2,4-D, 2,4,5-T and silvex [2,4,5-trichlorophenoxypropionic acid] brush killers). Not

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used on food crops, with the possible exception that some might be sprayed on pastures in areas immediately around brush. No residue problem exists (see 3) and no tolerances are necessary.

3. All 2,4-D and 2,4,5-T weed killers. Data have been presented to the U.S.D.A. that show no residue problem exists on small grain or in milk. In addition, the F.D.A. has agreed that there is no residue problem in meat. No residue tolerance is necessary.

4. Dow MCP Amine Weed Killer, (4-chloro-o-toloxyacetic acid, or 2-methyl-4-chlorophenoxyacetic acid, amine salt). The character and use of the chemical are similar to 2,4-D. It is the opinion of qualified experts in the field that no residue problem exists and that no tolerance will be necessary. An Industry-National Agricultural Chemicals Association Committee is now conducting analytical investigations.

5. Dalapon, Sodium Salt 85%, (sodium 2,2-dichloropropionate). Data have been presented to the U.S.D.A. that show no residue is present in sugar cane at harvest. With respect to pre-planting applications, dalapon is destroyed in warm, moist soil within several weeks after application (see DOWN TO EARTH, 11 (2):2 (1955)).

6. Dow General Weed Killer, Premerge and Dow Selective Weed Killer, (formulations based on dinitro-*o-sec*-butylphenol). Dinitro-*o-sec*-butylphenol has a zero tolerance. Dow has presented data to the U.S.D.A. that show no residues are present in the harvested crops when the products are used in accordance with label directions. These have been cleared by the U.S.D.A. It should be noted that in pre-harvest treatment of seed crops with Dow General Weed Killer, the label warns that the forage is not to be used as animal food, since such forage may contain DN residue.

7. Sodium TCA 90%, (sodium trichloroacetate). Data have been submitted to the U.S.D.A. that show no residues are present in sugar beets, cabbage and tomatoes when used in accordance with label recommendations. In view of the sugar cane data on dalapon, it is unlikely that residues will occur in this crop. Data are now being obtained. It is not anticipated that residue tolerance will be necessary for TCA.

INSECTICIDES

8. Arsenate of Lead. A residue tolerance of 7 ppm of combined lead has been set on the raw agricultural commodities that appear on the Dow label.

9. DDT. A residue tolerance of 7 ppm has been set on a wide variety of raw agricultural commodities appearing on the Dow label. An extension has been granted for meat until March 1, 1956.

10. *DN*-Dry Mix No. 1 (dinitro-o-cyclohexylphenol). A residue tolerance of 1 ppm on citrus has been set.

11. *DN*-Dry Mix No. 2 (dinitro-o-cresol). A zero tolerance has been set. No residues from strict dormant application.

12. *DN*-289 (dinitro-*o-sec*-butylphenol, triethanolamine salt). Zero tolerance. No residues from strict dormant application, or when applied after harvest.

13. *DN*-111 (dinitro-o-cyclohexylphenol, dicyclohexylamine salt). A 1 ppm tolerance has been set on apples, apricots, beans, blackberries, black-eyed peas, celery, cherries, citrus, grapes, loganberries, nectarines, peaches, pears, plums, quinces, raspberries and strawberries.

14. Lindane, BHC. Tolerances for a large number of raw agricultural commodities have been set as follows:

lindane	10 ppm
benzene hexachloride . .	5 ppm

An extension has been granted for lindane for several additional items until January 22, 1956 and on BHC in meat until March 1, 1956.

15. Lime Sulfur, Sulfur. The residues left by these pesticides are exempted from a tolerance.

16. Ovotran Wettable (p-chlorophenyl p-chlorobenzenesulfonate). An extension has been granted until March 1, 1956 for citrus. A petition has been submitted for tolerance in and on citrus, apples, pears, peaches, plums and prunes.

17. Parathion. A tolerance of 1 ppm has been set for a wide range of fruits and vegetables appearing on the Dow label. An extension has been granted to January 22, 1956 for field crops, forage crops, hops and olives.

18. Systox. A tolerance of 0.75 ppm has been set for apples, broccoli, brussels sprouts, cabbage, cauliflower, muskmelons, oranges, pears, potatoes, strawberries and walnuts.

19. Dowfume 75, Dowfume EB-5, Dowfume EB-15, Grain Fumigant (80-20 Mixture). All grain fumigants containing carbon tetrachloride, carbon disulfide, ethylene dichloride, and ethylene dibromide have been extended until March 1, 1956. Dow and other companies have been doing analytical work with the Food and Drug Administration to obtain the necessary data to establish tolerances or exemptions.

20. Methyl Bromide. An extension has been granted until January 22, 1956 for certain raw agricultural commodities. Dow has filed a petition asking for tolerances on apples, pears, quinces, onions, tomatoes, eggplants, beets, beans, cocoa beans, wheat, rice, rye, oats, barley, corn, grain sorghum, sweet potatoes, alfalfa hay, peanuts, potatoes, pecans, turnips, rutabagas and cottonseed.

21. Dowfume W-85, Dowfume W-40, Garden Dowfume (ethylene dibromide soil fumigants). An extension has been granted until January 22, 1956. Dow has filed a petition asking for tolerances on: Lima beans, corn, strawberries, sugar beets, asparagus, lettuce, sweet potatoes, parsnips, potatoes, turnips, rutabagas, carrots, celery and cottonseed. (Tobacco was not requested since it is not a food). Additional data are being obtained for tolerance in other crops.

FUNGICIDES

22. Lime Sulfur, Sulfur (See item 15).

23. Ferradow (ferbam). A tolerance of 7 ppm has been set for a wide variety of fruits and vegetables appearing on the Dow label.

OTHER AGRICULTURAL CHEMICALS

Nematocides, defoliants, and plant growth regulators are not defined as economic poisons by the Federal Insecticide, Fungicide, and Rodenticide Act. They are not registered by the U. S. D. A. and tolerances cannot be set under the Miller Amendment. Tolerances of 1 ppm naphthaleneacetic acid (App-L-Set) and 5 ppm for 2,4-D were set on certain fruits as a result of the 1950 hearings. Data are being obtained on Color-Set (2,4,5-trichlorophenoxypropionic acid) to determine if residues are present at harvest. Magron (Dow magnesium chlorate defoliant) has been investigated and no residues of magnesium chlorate have been found on rice or beans defoliated with this product.

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ESTERO

Containing 6.15 Pounds per
FOR THE

Active Ingredient: 2,4,5-Trichlorophenoxyacetic Acid, Propylene Glycol (C_3H_6O to $C_9H_{18}O_3$) Butyl Ether Esters	**65.3%**
2,4,5-Trichlorophenoxyacetic Acid Equivalent	**42.5%**
Inert Ingredients	**34.7%**

Esteron 2-45 is recommended for use in controlling herbaceous and woody plants growing in rangeland, pastures, fence rows, ditch banks, farmyards and right-of-ways. It is effective in controlling certain 2,4-D resistant plants such as ash, brambles, ground cherry, hawthorn, horse nettle, maple, mesquite, oak, osage orange, palmetto, poison ivy, prickly pear cactus, salmonberry, wild blackberry, wild rose and certain species of Ribes, as well as many other woody and herbaceous species.

DIRECTIONS

PREPARING THE SPRAY: Add half the required amount of water or oil to the spray tank, then add the Esteron 2-45 with agitation, and finally the balance of the water or oil with continued agitation. **WARNING:** If Esteron 2-45 is to be used in preparing straight oil mixtures, do not let water get into the Esteron 2-45 itself nor into the finished mixture. **NOTE:** Esteron 2-45 forms an emulsion—not a solution—in water, and the Esteron 2-45 tends to separate out on standing. Provide agitation to prevent such separation and ensure uniformity of spray mixture.

For Treating Small Areas: One tablespoonful of Esteron 2-45 in 1½ gallons of oil or water is approximately equivalent to one quart in 100 gallons.

FOLIAGE TREATMENT: Spray woody growth up to 6 or 8 feet tall after foliage is well developed, using a drenching spray containing 3 quarts of Esteron 2-45 per 100 gallons of water. Taller brush can be sprayed successfully, although in many cases basal bark or stump treatment is preferable. Poison ivy, most brambles and some other species may be controlled by using 2 quarts per 100 gallons of water. **Coverage should be complete, and all parts of the plants, including foliage, shoot stems and bark, should be wet with the spray.** Best results usually will be obtained from applications made soon after maximum foliage development in the spring. With good growing conditions and adequate soil moisture, applications may be made up to 2 or 3 weeks before normal frost date. Less effective control may result during hot, dry weather when deep soil moisture is deficient. Power equipment with pressures up to 250 pounds will aid

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ON® 245 TH BR

Gallon of Powerful, Low Volatility Esters of 2,4,5-T • 2,4,5-T Acid Equival

CONTROL OF MANY SPECIES OF TREES, BRUSH AND BROAD-LEAVED W

In obtaining satisfactory spray coverage. Repeat applications may be necessary as new growth develops, but a single treatment in any one year is usually sufficient. **NOTE:** Many broad-leaved weeds are controlled by this application.

BASAL BARK TREATMENT: Brush and small trees can be controlled by spraying the basal parts of brush stems and tree trunks to a height of 12 to 15 inches from the ground line. Use a solution of 4 gallons of Esteron 245 in 96 gallons (1 pint in 3 gallons) of diesel oil, fuel oil or kerosene. With certain species, 2 gallons of Esteron 245 in 98 gallons of diesel oil, fuel oil or kerosene is effective. Knapsack or power equipment may be used, **but complete wetting of the indicated area is necessary, particularly at the ground line. This means spraying until run-down or run-off to the ground line is noticeable. Old or rough bark requires more spray volume than young or smooth bark.** Low pressures are desirable. Apply at any time, including the winter months. Often delayed response and killing can be expected. Treated brush or trees preferably should not be cut for a period of one year following application.

STUMP TREATMENT: Where growth is more than 6 to 8 feet tall, cut it close to the ground and spray the stumps and stubs with 4 gallons of Esteron 245 in 96 gallons (or 1 pint in 3 gallons) of diesel oil, fuel oil or kerosene, mixed thoroughly. Wet thoroughly **all exposed bark, as well as cut surfaces. This means spraying until run-down or run-off to the ground line is noticeable. Old or rough bark requires more spray volume than young or smooth bark.** Apply at any time, including the winter months, preferably to freshly-cut stumps. Best results are usually obtained on stumps two inches across or larger.

"FRILL" TREATMENT: For large trees, make a single-hack girdle or "frill" of overlapping axe cuts completely around the tree as close to the ground as possible. Treat the injured area with a mixture of 2 gallons of Esteron 245 in 100 gallons (1½ pint in 3 gallons) of diesel oil, fuel oil or kerosene.

AIRPLANE APPLICATIONS: To control mesquite, use 1 to 1½ pints of Esteron 245 per acre in 3 gallons of water and 1 gallon of water-clear diesel fuel. Apply 50 to 80 days after first leaves appear. Do not treat if drouth has prevented heavy foliage growth. For **post oak and blackjack oak,** use 2 quarts of Esteron 245 per acre. Mix this either with 3 to 4 gallons of water and 1 gallon of diesel oil, or with 3 to 4 gallons of diesel oil. Apply after foliage is fully developed. **NOTE:** Soil moisture must be adequate for normal growth. Re-treat as necessary in succeeding years.

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MAY 1139026

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ON® 245

THE MODERN MULTI-USE BRUSH AND WEED KILLER

Gallon of Powerful, Low Volatilty Esters of 2,4,5-T • 2,4,5-T Acid Equivalent 4 Pounds per Gallon

E CONTROL OF MANY SPECIES OF TREES, BRUSH AND BROAD-LEAVED WEEDS

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WARNING!

Do not apply Esteron 245 directly to, or otherwise permit it to come into contact with vegetables, flowers, grapes, fruit trees, ornamentals, cotton or other desirable plants which are sensitive to 2,4,5-T, and do not permit spray mists containing it to drift onto them, since even minute quantities of the spray may cause severe injury during both growing and dormant periods. (Coarse sprays are less likely to drift.) Accordingly, **applications by airplane, ground rigs and hand dispensers should be carried out only when there is no hazard from drift. Do not apply by airplane in the vicinity of cotton, grapes or other desirable 2,4,5-T susceptible vegetation.** At higher temperatures vaporization may cause injury to susceptible plants growing nearby.

Do not use on lawns of creeping grasses, such as bent, except for spot spraying, nor on freshly seeded turf until grass has become well established. (Most legumes are usually damaged or killed.) Do not contaminate irrigation ditches or water used for domestic purposes. Do not store near fertilizers, seeds, insecticides or fungicides. To avoid injury to desirable plants, do not store, handle or apply other agricultural chemicals with the same containers or equipment used with Esteron 245.

Local conditions may effect the use of herbicides. State agricultural authorities in many states issue recommendations to fit local conditions.

Be sure that use of this product conforms to all applicable regulations.

CAUTION! MAY CAUSE SKIN IRRITATION
Avoid Contact with Eyes, Skin and Clothing

NOTICE

Seller makes no warranty of any kind, express or implied, concerning the use of this product. Buyer assumes all risk of use or handling, whether in accordance with directions or not.

U. S. Patents No. 2,390,941; 2,396,513; 2,472,347 and 2,562,855

THE DOW CHEMICAL COMPANY
MIDLAND, MICHIGAN
MIDLAND DIVISION

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increased number of diskings between spraying and seeding resulted in a slightly lower stand of trefoil plants. This was not apparent at the Southeastern Ohio test. In other words, a lesser amount of tillage on the 7-day interval left a firmer seedbed which meant less coverage of seeds and earlier germination. Current tests in Ohio are directed at determining the minimum amount of tillage necessary with herbicide treatment.

TABLE II

PERCENT STAND OF KENTUCKY BLUEGRASS IN TILLED AND UN-TILLED AREAS REMAINING IN THE FALL OF 1956 FOLLOWING HERBICIDE TREATMENTS MADE ON OCTOBER 20, 1955 AND APRIL 19, 1956

Herbicide Rate lb./A Acid Equivalent	*Fall Applied*		*Spring Applied*	
	Tillage	*No-tillage*	*Tillage*	*No-tillage*
Dalapon - 10	Trace	75%		
Dalapon - 5			Trace	45%
Dalapon - 20	Trace	35%		
Dalapon - 10			Trace	Trace
ATA - 4	Trace	90%		
ATA - 2			Trace	30%
ATA - 8	Trace	60%		
ATA - 4			Trace	10%

Table II summarizes data taken in a field test at the U.S.D.A. Soil and Water Conservation Research Station at Coshocton, Ohio. Duplicate plots were laid out in the fall of 1955 for spraying to compare fall and spring treatments. Estimates of stand of bluegrass in the 1955 fall and 1956 spring treated areas were made in the fall of 1956. The main point brought out in these data is the effect of tillage on percent of bluegrass kill, both fall and spring. The tillage implement used in this case was a disk harrow. All tillage plots were disked thoroughly 5-10 days after spraying and fall sprayed plots were disked again in the spring prior to seeding. The bluegrass kill was almost complete on fall and spring sprayed tillage plots. The heavier fall herbicide rates without any tillage also resulted in almost complete kill of bluegrass. The importance of tillage in conjunction with spring applied chemicals is quite apparent even at heavier rates.

The stand of trefoil obtained by band seeding in this test was variable and inconclusive but indications are that satisfactory stands of trefoil can be established with fall applied herbicide and no cultivation. On these fall-treated, no-tillage plots the trefoil stand was estimated to be from 50 to 80 percent. Again, winter survival will affect appraisals of results.

In conclusion these data show the possibilities of using herbicides in seedbed preparation for birdsfoot trefoil. Proper use of available materials will suppress competing vegetation sufficiently to permit better stands of trefoil with a minimum of cultivation. Dalapon can be used effectively as either a spring or fall treatment. It lends itself readily to spring application because of its low toxicity to birdsfoot trefoil seedlings. On the other hand ATA is probably best applied only in the fall if spring seeding is intended. There is need for further research to determine: (1) The minimum amount of tillage necessary, (2) The usefulness of the new pasture renovation seeding equipment that is on the market, (3) Minimum amount of chemical to use under various conditions.

REFERENCES

(1) Sprague, M. A., The substitution of chemicals for tillage in pasture renovation, Agron. Jour. 44:405-409, 1952.
(2) Kates, Allan H., M. A. Sprague and R. J. Aldrich, Promising chemicals for seedbed preparation, Down To Earth, Vol. 10, No. 4, 1955 (Pages 10 and 11).

RESIDUE TOLERANCES FOR DOW AGRICULTURAL CHEMICALS

By G. E. LYNN, The Dow Chemical Company*

It is now well recognized that pesticidal chemicals must be used in agriculture to prevent crop and animal losses during production and storage. Pesticidal chemicals, such as insecticides and weed killers, have become a necessary part of the farming economy and take their place in importance along with the tractor and other modern farming tools. Pesticides can be used safely and without hazard to the consumer of the food crop. Although some pesticides may leave trace residues on food crops, it has been shown by extensive toxicological investigations that the small amounts of most pesticides that appear in food can be consumed without any adverse effect on the health of man or animals. Under these circumstances the Miller Amendment to the Federal Food, Drug and Cosmetic Act provides a means by which adequate residue tolerances on raw agricultural commodities can be established to permit the use of the pesticides. The Dow Chemical Company has obtained the necessary data to establish many such tolerances for its agricultural chemical products. Additional data are being obtained to expand the list of commodities and crops on which both old and new pesticides may be used.

Many pesticidal chemicals are used in ways which do not result in a residue. Application early in the growing season, or use at low dosage rates often eliminates the residue problem. Numerical tolerances are not necessary under these circumstances. These chemicals are, however, subject to registration and ample proof of the lack of residue must be submitted to the United States Department of Agriculture before a registration is granted. For example, Premerge†, containing dinitro-*o*-sec. butylphenol, is recommended for a variety of pre- and postemergence herbicidal uses. Each use has been investigated thoroughly not only for effectiveness on weeds and safety on crops but also for possible crop residue. When used as directed on the label, there is no crop residue problem.

Users of agricultural chemicals are urged to follow the directions that appear on the labels. Any other use, or departure from label (*Continued next page*)

*Midland, Michigan.

† Premerge is a trademark of The Dow Chemical Company.

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recommendations, may result in poor performance and residue problems not covered by tolerances.

Some chemicals, used in agriculture, are not classified as pesticides by the Federal Law and thus cannot be registered. Dow agricultural chemicals in this category are Magron, Colorset, App-I-set, anhydrous ammonia and the soil fumigants, Fumazone, Telone and Dorlone. The Dow Chemical Company has carefully investigated these chem cals and when each is employed in accordance with goc agricultural practice, as directed on the label, no publ health hazard will result.

The following information summarizes the status many registered Dow Agricultural Chemical products as January 15, 1957.

RESIDUE TOLERANCE STATUS

FUMIGANTS

Dow Agricultural Product	*Active Ingredients*	*Tolerances*
Dowfume* EB-5 and Dowfume EB-15	ethylene dibromide ethylene dichloride carbon tetrachloride	Carbon tetrachloride, ethylene dichloride, carbon disulfide, and the orga bromide residues from ethylene dibromide are exempted from the requ ment of a tolerance when used as a fumigant for the following grains: barley, corn, oats, popcorn, rice, rye, sorghum (milo) and wheat. Tolerances of 50 ppm are established for residues of inorganic bromi (calculated as Br) in or on the following grains that have been fumiga with ethylene dibromide: barley, corn, oats, popcorn, rice, rye, sorghum (milo) and wheat. Sulfur dioxide in amounts used does not constitute a residue problem.
Dowfume 75	ethylene dichloride carbon tetrachloride	
Dowfume 80-20	carbon tetrachloride carbon disulfide	
Dowfume C	carbon tetrachloride carbon disulfide ethylene dibromide	
Dowfume F	carbon tetrachloride ethylene dichloride ethylene dibromide sulfur dioxide	
Dowfume G	ethylene dichloride carbon tetrachloride ethylene dibromide	
Dowfume H	carbon tetrachloride carbon disulfide sulfur dioxide	
Dowfume J	ethylene dichloride carbon tetrachloride ethylene dibromide	
Vertifume*	carbon tetrachloride carbon disulfide	
Serafume*	carbon tetrachloride ethylene dibromide ethylene dichloride carbon disulfide	
Methyl Bromide	methyl bromide	The tolerances for residues of inorganic bromides (calculated as Br) or on raw agricultural commodities which have been fumigated with met bromide are as follows: (a) 5 ppm in or on apples, pears, quinces. (b) 20 ppm in or on eggplants, onions, tomatoes. (c) 30 ppm in or on beets, rutabagas, turnips. (d) 50 ppm in or on alfalfa, hay, barley, beans, green beans, lima beans, snap beans, corn, grain sorghum (milo), oats, rice, rye, wheat. (e) 75 ppm in or on potatoes, sweet potatoes. (f) 200 ppm in or on cottonseed.
Dowfume MC-2	methyl bromide chloropicrin	When used in accordance with directions as a plant bed treatment and w transplants are moved to soil not treated with this fumigant, no detect residues are probable in or on a food crop.
Dowfume W-85	ethylene dibromide	The tolerance for residues of inorganic bromide (calculated as Br) in or raw agricultural commodities grown on soil treated with ethylene dibrom are as follows: (a) 5 ppm in or on lima beans, strawberries. (b) 10 ppm in or on asparagus, cauliflower. (c) 25 ppm in or on cotton. (d) 50 ppm in or on sweet potatoes. (e) 75 ppm in or on carrots (with or without tops), parsnips.
Picfume*	chloropicrin	Leaves no residues on food crops when used as a soil fumigant in accord with Dow label instructions.

* Trademarks of The Dow Chemical Company

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HERBICIDES

Dow Agricultural Product	*Active Ingredients*	*Tolerances*
Dowpon*	dalapon, sodium salt (2,2-dichloropropionic acid, sodium salt)	Tolerances established for 5 ppm in or on sugar beets and 35 ppm in or on cottonseed (calculated as 2,2-dichloropropionic acid). No residues result from early pre-planting treatments when used in accordance with Dow label instructions. No residues result from use on sugar cane.
Dow Sodium TCA 90%	trichloroacetic acid, sodium salt	When used in accordance with Dow label recommendations, no residue in or on a food crop will result.
Premerge*	dinitro-*o*-sec butylphenol, alkanolamine salts	Zero tolerances established. When used in accordance with Dow label recommendations, no residues in or on a food crop will result.
Dow Selective Weed Killer	dinitro-*o*-sec-butylphenol, ammonium salt	
Dow General Weed Killer	dinitro-*o*-sec-butylphenol,	
Esteron* 44	2,4-dichlorophenoxyacetic acid, isopropyl ester	When used in accordance with Dow label recommendations, no residue in or on a food crop will result. (Note: A tolerance of 5 ppm 2,4-dichlorophenoxyacetic acid has been established in or on apples, citrus fruits, pears, and quinces.)
Esteron Ten Ten Esteron 99	2,4-dichlorophenoxyacetic acid, propylene glycol butyl ether esters	
Esteron 76E	2,4-dichlorophenoxyacetic acid isopropyl ester and 2,4-dichlorophenoxyacetic acid, butyl esters	
Esteron Brush Killer	2,4-dichlorophenoxyacetic acid, propylene glycol butyl ether esters, and 2,4,5-trichlorophenoxyacetic acid, propylene glycol butyl ether esters	
Esteron 245 Reddon*	2,4,5-trichlorophenoxyacetic acid, propylene glycol butyl ether esters	
Dow 2,4,5-T Amine Weed Killer	2,4,5-trichlorophenoxyacetic acid, triethylamine salt	
Kuron*	2(2,4,5-trichlorophenoxy) propionic acid, propylene glycol butyl ether esters	
2,4-Dow Weed Killer, Formula 40 Home Use Weed Killer	2,4-dichlorophenoxyacetic acid, alkanolamine salts	
Dow MCP Amine Weed Killer	2-methyl-4-chlorophenoxyacetic acid, alkanolamine salts	

* Trademarks of The Dow Chemical Company

INSECTICIDES

Dow Agricultural Product	*Active Ingredients*	*Tolerances*
DN-Dry Mix* No. 1	dinitro-*o*-cyclohexylphenol	A residue tolerance of 1 ppm is established in or on citrus fruits.
DN-111*	dinitro-*o*-cyclohexylphenol, dicyclohexyl amine salt	A residue tolerance of 1 ppm is established in or on apples, apricots, beans, blackberries, black-eyed peas, celery, cherries, citrus fruits, grapes, loganberries, nectarines, peaches, pears, plums, (fresh prunes), quince, and raspberries.
DN-Dry Mix* No. 2	dinitro-*o*-cresol	Zero tolerance established. When used in accordance with Dow label recommendations, no residue in or on a food crop will result.
DN-289*	dinitro-*o*-sec. butylphenol, tri-ethanolamine salt	Zero tolerance established. When used in accordance with Dow label recommendations, no residue in or on a food crop will result.
Ovotran* Wettable	ovex (p-chlorophenyl p-chlorobenzenesulfonate)	Residue tolerances are established as follows: (a) 3 ppm in or on apples, peaches, pears, and plums (prunes). (b) 5 ppm in or on grapefruit, lemons, oranges, and tangerines.
Dowicide* A	Sodium-*o*-phenylphenate	A tolerance of 10 ppm is established for residues of sodium *o*-phenylphenate (calculated as *o*-phenylphenol) in or on each of the following citrus fruits: oranges, lemons, limes, grapefruit, tangerines, tangelos, citrus citron, kumquat. A tolerance of 5 ppm is established for residues of sodium *o*-phenylphenate (calculated as *o*-phenylphenol), in or on apples and pears.

* Trademarks of The Dow Chemical Company

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ACD file no. HG-51

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TOXICITY OF HERBICIDES TO DOMESTIC ANIMALS

by

T. A. Hymas, DVM
Agricultural Chemical Research
THE DOW CHEMICAL COMPANY
Midland, Michigan

May 29, 1958

In reviewing the paper that I am about to present I believe the title should more appropriately be "The Lack of Toxicity of Herbicides to Domestic Animals" rather than "Toxicity of Herbicides to Domestic Animals."

The use of herbicides for the control of undesirable vegetation has been practiced for many years, some of the older materials that have been used are the chlorates, arsenicals, boron compounds, certain oils, and even table salt. While some of these materials are still being used on occasion for this purpose, research over the past 14 years has revealed new herbicide materials that are superior to the older materials and in some instances are less toxic to animals and also man. The herbicides that I wish to discuss at this time are organic chemical compounds that act systemically when applied to vegetation. They have a selective toxicity to specific plants and are currently being used throughout the world for the control of undesirable vegetation.

I am going to confine my remarks to several of the 2,4-D type materials which can be classed as chlorinated phenoxy-acetic acid compounds, and to a relatively newcomer to the field of selective herbicides, dalapon, known chemically as 2,2-dichloropropionic acid.

To begin, I would like to give some comparative data that has been obtained by numerous investigators, to compare the toxicity of these herbicides when given orally to different species of animals.

In Table No. 1 the acute oral LD_{50} values for several species of animals fed the most widely used chlorophenoxy-acetic acid compounds is given. These are 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxy-acetic acid), and silvex which is 2-(2,4,5-trichlorophenoxy) propionic acid. The chlorinated phenoxyacetic and propionic acid compounds are very similar in chemical make-up, they are also similar in their toxicity to animals as one might expect. Dogs appear to be the most susceptible species to the chlorinated phenoxy acetic and propionic acid materials. Where there are two sets of figures given in the table they represent a range of values to cover the pure acids, also esters and salts of the compounds, sex differences, and work by different investigators. While the basic acid has not been greatly altered when formulated, as an ester or salt, the formulation may only slightly alter the biological activity of the toxicant as measured by herbicidal activity and toxicity to animals.

The value given for cattle was obtained using a mixture of 2,4-D and 2,4,5-T.

Because of the similarity of toxicity of 2,4-D and 2,4,5-T to laboratory animals and the wide use of the combination of these herbicides in commercial products, it was decided to use a combination of the two materials (Esteron* Brush Killer) containing approximately equal parts of 2,4-D and 2,4,5-T as esters, to study the acute oral toxicity of the materials in cattle.

The cattle used for these studies were dairy cattle of mixed breeding weighing from 250 to 336 kilograms. Table No. 2 is a summary of the work accomplished.

Experiment #1: A steer weighing 291 Kg. was given a single oral dose of 1,000 mg/Kg acid equivalent of Esteron Brush Killer, sufficient herbicide to prepare 11 gallons of spray at the highest level recommended, 4 qts/100 gal. of spray, or 16.6 gallons at the usual recommended rate of 3 qts/100 gal. of spray. This dosage had no observable effect on the steer.

A second steer weighing 295 Kg. was given 1,000 mg/Kg acid equivalent of this same mixture of 2,4-D and 2,4,5-T, as Esteron Brush Killer, on each of three successive days. The quantity of herbicide given would treat 1/2 acre of heavy brushy, woody, or weedy area at the recommended use level. The day following the third dose a general depression was noted, the animal was off feed and lethargic. The general attitude and behavior became progressively worse until the animal died on the third day following the last dose. Death was undramatic. The steer evidenced no pain, no struggling, or signs of violence. Upon autopsy it was noted that the rumen had a strong odor that is characteristic of the 2,4-D type materials. The reticulum, rumen, omasum, abomasum and fore-part of the duodenum were abnormally dry and impacted. Rumen stasis was evident. Beyond the duodenum the G. I. tract contents were fluid. No other gross abnormalities were evident.

A third steer weighing 295 Kg. was given 500 mg/Kg on each of two consecutive days. On the third day the animal was off feed and rumen motility had essentially ceased. Otherwise the animal appeared normal. On the fourth day, this steer appeared normal and there were no discernable after effects.

A fourth animal weighing 336 Kg. was given 500 mg/Kg on each of three successive days and no toxic symptoms were observed. The quantity of herbicide given would make 27.5 gallons of spray at the recommended concentration of 4 lbs. acid equivalent per 100 gallons of spray. The animal remained on full feed without showing effects of any kind.

A fifth steer weighing 250 Kg. was then given 100 mg/Kg of the 2,4-D, 2,4,5-T mixture on 15 consecutive days and observed carefully. There were no outward appearances of adverse effects. The animal was necropsied 48 hours following the last dosage. Gross examination of the internal organs revealed only slight petechial hemorrhage in the duodenum and a very mild diffuse irritation in the abomasum. Histological examination of tissues revealed very mild pathology in the liver and kidneys. The liver contained small areas of focol hemorrhagic necrosis surrounded by areas

* Trademark of The Dow Chemical Company

of fatty degeneration. In the kidneys a slight interstitial adema and congestion of the cortical medullary regions was observed.

The amount of 2,4-D and 2,4,5-T that was ingested by this steer was 0.8 lb., which is sufficient to make 20.5 gallons of spray to treat dense or hard to kill foliage or herbage.

It would be impossible for one or more bovine to consume all of the toxicant applied in 20 to 30 gallons of spray even if they were confined in an area where all vegetation was treated. Some toxicant drips or is sprayed on the ground and much vegetation that is sprayed is of an unpalatable nature being brush, weeds or the like. I do not believe the chlorinated phenoxy acetic acid herbicides constitute any hazard whatsoeve to cattle if they are handled with reasonable care and used according to recommendations of the manufacturer.

I would now like to consider dalapon (2,2-dichloropropionic acid) for a few minutes.

Slide #3 gives the acute oral toxicities of dalapon in several species of animals. In evaluating these figures it is well to remember that the LD_{50} value for sodium chloride (table salt) is 2,500 to 3,500 mg/Kg in laboratory animals. The figure reported for dogs, an LD_{50} of greater than 1,000 mg/Kg, is the largest dosage that dogs would retain without emesis. We have found that quite often it is very difficult to determine acute oral toxicities in dogs because of their ability to regurgitate such materials and our inability to control this reflex even with the use of morphine, various sedatives, tranquilizers, etc., which, of course, we are always reluctant to use because of the effect of the anti-emetic itself.

I would like to say at this point that where we have had an opportunity to compare the acute oral toxicity values of herbicide materials in small animals with those obtained using cattle, the two values are usually close. If real differences exist, to date, in our work, cattle have tolerated the larger doses, perhaps because of the bacterial flora in the rumen and their ability to decompose organic materials.

We attempted to attain acute oral LD_{50} values for dalapon in cattle but, as can be seen in slide #4, when 4,000 mg/Kg, approximately 3.5 lbs. of the chemical was "pumped" into a steer at one time, 5.5 lbs. was "pumped" into another steer over a ten day period and an equal amount on a weight basis "pumped" into a suckling calf, and the worst reaction seen was a transient digestive upset and, I might add, a very effective grass killing job in our grass pasture where urination occurred following dosing. We gave up trying to kill cattle with dalapon as it appeared they would need to be drowned in it. Histopathological studies of tissues from the animals that were sacrificed revealed no abnormalities. We concluded that dalapon was not a hazard on farms, rights-of-way, in ditches, or farm ponds, or under any conditions of practical use. This conclusion has been substantiated by several years use in this country and other countries without a single substantiated case of poisoning being brought to our attention. And, we believe if poisoning cases had occurred we would know about them. Being associated with a chemical company for several ye

I know that where chemicals are used and any abnormality is observed, the first thought is usually--the chemical did it. Any legitimate chemical manufacturers has always expended considerable time and effort to determine the hazards of having chemicals used where men and animals are likely to be exposed.

A great deal of work and effort always goes into toxicology work on the product also the preparation of labels and literature to inform the consuming public of the limitations, handling hazards and possible hazards that may be encountered when the chemical is used.

Dalapon is being widely used to control undesirable grass species such as Johnsongrass, quackgrass, and is also being used to control the desirable species of grass that are growing in undesirable locations. It is also finding wide usage to control cattails and marsh grasses that so commonly choke drainage ditches, waterways, farm ponds, wildlife refuges, etc. The active material may be applied at a rate of from 1.5 to 40 lbs. per acre depending on the type of problem being attacked. When the material is prepared as a spray up to 20 lbs. per 100 gallons, a 1,000# cow would have to drink at least 15 gallons of the undiluted most concentrated spray mix to evidence the slightest symptoms of intoxication. And she could probably survive even a dose of double this quantity or 30 gallons of the 20 lbs. per 100 gallon spray mix. If a 1,000# cow had access to a spray tank containing the usual and most frequent concentration of dalapon, 10-15 lbs. per 100 gallons, she would need to drink 33 to 50 gallons of the spray mix to evidence the slightest intoxication.

It is interesting to note that since the advent of the 2,4-D herbicides, over 225 million lbs. of the herbicide have been used. This would be sufficient to treat some 565 million acres of land at a practical use level of 0.4 lbs. acid equivalent per acre. To date, to our knowledge, there has not been one single substantiated case of poisoning due to the herbicide. As you gentlemen might guess, there have been numerous allegations and there have been a number of cases of reported poisonings that have occurred following application of herbicides, but in every case that has been followed up and a diagnosis was possible, the herbicide has been exonerated.

There has been much discussion of the possibility that spraying with herbicides might cause some plants to become toxic and other plants that are ordinarily non-palatable to become palatable. Considerable investigation of these possibilities has resulted in the general conclusion that from a practical standpoint these suppositions are more fanciful than factual. To date the only effect on herbage that has been proven that might be harmful to livestock is in the case of certain plants that have been sprayed with 2,4-D or similar materials. The nitrate content can be increased. For instance, in the case of sugar beets, accidentally sprayed with 2,4-D, the nitrate content of the leaves is actually increased and it is possible that the feeding of sugar beet tops treated with 2,4-D could cause nitrate poisoning. Of course, the only time 2,4-D would be applied to sugar beets is by accident, as the material is not recommended for use in sugar beet production and has never been applied for thinning purposes. It has been reported that other plants, such as lambsquarters, pigweed,

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and smartweed will increase their nitrate content after treatment with 2,4-D. Since livestock do not ordinarily eat these species, it would not seem to be a practical problem, although it is possible that if animals were starved to eating such plants and the plants were sprayed with 2,4-D that nitrate poisoning could occur. It might be pointed out that environmental and mechanical factors can cause as significant changes in nitrate level as herbicides.

The question has been raised many times: Will wild cherry present a special problem when sprayed with 2,4-D type herbicides? It has been rather conclusively proven by Grigsby and Baugh, also Grigsby and Blakeslee at the Michigan State University, also by Barrons and Lynn of The Dow Chemica Company that wild cherry does not increase in HCN content when it is treated with 2,4-D. This has been proven by chemical methods and further substantiated by practical type tests. In a test conducted by Grigsby and Blakeslee of the Michigan State University and L. L. Coulter of The Dow Chemical Company, sheep were pastured on wild cherry that had been sprayed. Immediately prior to the time the sheep were turned into the brushy pasture, two quartersections of the pasture including the wild cherry plants were sprayed with a mixture of 2,4-D and 2,4,5-T, the sheep grazed the wild cherry plants--not only at the time they were turned into the pasture but for several days following, and until the vegetation actually turned yellow and brown, and was so markedly affected by the herbicide that it was no longer palatable. At no time was there any adverse effects noted in these sheep, and at no time was there evidence that the treatment of the herbage affected the normal habits or the normal functions of the sheep that grazed on it. There was no preference shown for either treated or untreated plants.

In all of the alleged cases of herbicidal poisoning that have been definitely diagnosed, the diagnosis has been such causes as: shipping fever, leptospirosis, anaplasmosis, arsenic, poisonous plants, lead, poor marksmanship (hot lead), parasites, hardware disease, etc. It should be born in mind that many types of weeds and foliage have in the past and will continue to cause poisoning whether they are sprayed or whether they are not sprayed with herbicides. It is also known that various members of the sudan grass and sorghum family have caused difficulties in the past and undoubtedly will cause them in the future. Here, again, losses occurred prior to the use of modern herbicides and will continue despite herbicide usage.

Some workers have felt that these systemic herbicides may improve the palatability of normally unpalatable poisonous plants to a degree where animals will graze them. However, in actual grazing trials on desirable grasses and legumes, livestock have never shown preference for the treated foliage. In fact, where there has been a preference shown, it has always been for the untreated plants, and until it can be proven that the palatability of any plants is actually increased by the use of herbicides, we should keep an open mind.

I have prepared a table (Slide #5) to show the minimum and maximum spray concentration of the products used and the minimum quantities of spray a 1,000# cow would need to drink, or sprayed area she would have to consume all of the vegetation from (assuming all spray is retained on the vegetation) to evidence even mild intoxication from ingestion of the herbicide.

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You should remember that the more concentrated the spray, the heavier and more unpalatable the vegetation to be sprayed.

I have brought along a few slides to give you a better idea of just how these materials work and where you might expect to see them used.

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Table #1

ACUTE ORAL TOXICITIES OF
2,4-D AND RELATED COMPOUNDS

LD_{50} (Mg/Kg)

Animal Species	2,4-D	2,4,5-T	Silvex
Mice	375-713	389-551	1410
Rats	375-805	481-500	600-621
Rabbits	424-800	712	750-819
Guinea pigs	469-1000	381-750	1200-1250
Dogs	100	100	--
Chicks	540-2000	310	1190
Cattle		>1000*	

* Mixture of equal parts 2,4-D and 2,4,5-T

Table #2

TOXICITY OF A MIXTURE OF
2,4-D, 2,4,5-T GIVEN ORALLY TO CATTLE

Sex and Wt. in Kgs.	Dosage Mg/Kg	Observations
S-291	1000	No effect
S-295	1000/da. for 3 da.	Fatal
S-295	500/da. for 2 da.	Moderate transient toxicity
S-336	500/da. for 3 da.	No effect
S-250	100/da. for 15 da.	Grossly no effect. Microscopically, liver & kidney mildly affected.

Table #3

ACUTE ORAL TOXICITY OF DALAPON
(Sodium Salt)

Animal Species	LD_{50} Values
Mice	>4,000
Rats	6,600-8,100
Guinea pigs	3,400
Rabbits	3,400
Dogs	>1,000
Chicks	5,700
Cattle	>4,000

Slide #4

TOXICITY OF DALAPON (Sodium Salt)
GIVEN ORALLY TO CATTLE

Sex and Wt. in Kgs.	Dosage Mg/Kg	Observations
S-336	2,000	No effect
S-290	3,000	Mild transient toxicity
S-337	3,500	Mild transient toxicity
S-394	4,000	Mild transient toxicity
F*-252	1000/da. for 10 da.	Moderate toxic symptoms
S*-59	1000/da. for 10 da.	No effect

* Necropsied for histopathology studies

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Slide #5

Herbicide	Spray Concentration lbs/100 gal.	1000# Cow May Become Intoxicated if She Ingests Spray Concentrate (Gallons)	1000# Cow May Become Intoxicated if She Ingests All Spray from Sprayed Area (Sq. feet)
2,4-D and 2,4,5-T	0.25	400	174,240
	4.0	25	10,890
Dalapon (Sodium salt)	1.5	333	143,748
	20.0	25	5,445

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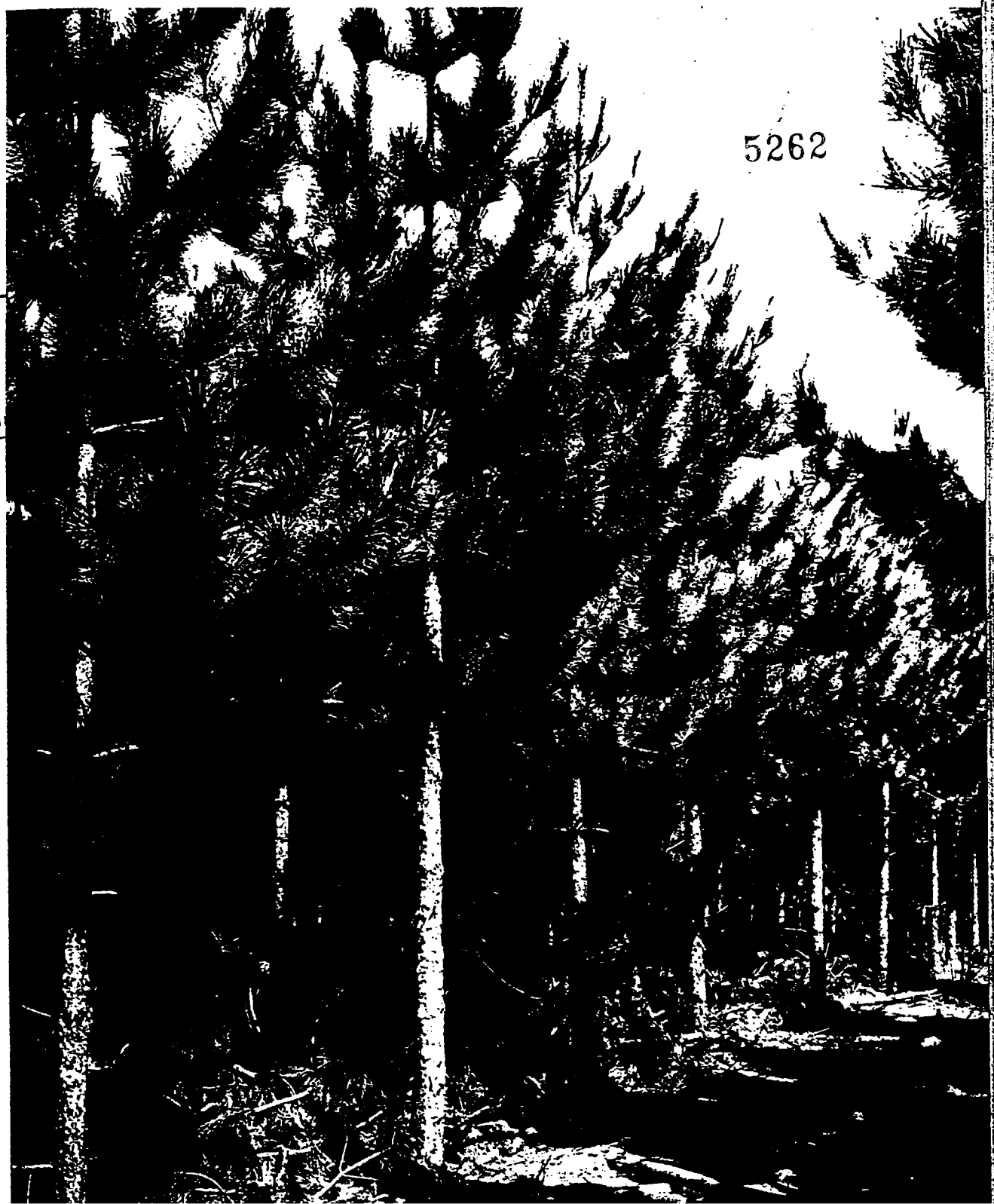
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Basal Spray with 2,4,5-T for Winter Hardwood Control In East Texas

Removal of competing hardwoods is essential to re-establishment of pine on many dry sites. Aerial spraying of silvicides or land-clearing with heavy machinery promises to be useful on extensive operations. For owners of small tracts, however, hand methods that can be applied at planting time are most acceptable.

Search for such a method at the Austin Experimental Forest in east Texas revealed that for December application, and, presumably throughout the dormant season, 2, 4, 5-T is more effective when applied as a basal spray than when painted on cut stumps.

Triplicate sets of plots were treated and planted to loblolly pine (*Pinus taeda* L.) in December 1953, near the beginning of the normal planting season. On one set of plots all hardwoods were basal-sprayed, on a second set all hardwoods were cut close to the ground, and on the third the hardwoods were similarly cut and the stumps painted with silvicide. The silvicide for the first and third treatments was a mixture of 1 gallon of Dow Esteron 245 (a propylene glycol butyl ether ester) in 16 gallons of No. 2 diesel oil, a concentration of 23.5 pounds of acid per hundred gallons. In both treatments the solution was applied to the point of runoff.

Of the original stand of 13-year-old mixed hardwoods, 20 sweetgum (*Liquidambar styraciflua* L.) trees or stumps on each plot were observed for three years. By April 1954, 92 percent of the stumps without silvicide had sprouted, and this increased to 100 percent by the following November. The 2, 4, 5-T solution on cut stumps delayed but did not reduce sprouting: 37 percent of the stumps sprouted by April, 90 percent by November, and 97 percent by April 1955. Untreated stumps produced significantly larger and slightly more sprouts than stumps with silvicide, but by the third growing season sprouts on both treatments had outgrown the surviving pine seedlings.

Ninety-three percent of the basal-sprayed trees eventually died, though more than half of them retained their leaves throughout the first growing season. Only 5 percent of the sprayed trees sprouted.

Relative effectiveness of the treatments as site preparations is revealed by survival of planted loblolly pine seedlings. In November 1954, after a very dry summer, loblolly survival was 53 percent on the plots where silvicide was applied on stumps, 55 percent where stumps were left untreated, and 83 percent where hardwoods had received basal spray. The silivicide had no adverse effect on the pine seedlings under either treatment, even when planting followed its application by only a few hours.

However, none of the three methods afforded an adequate "one shot" release treatment for Texas conditions. After three growing seasons, two of which were very dry, pine survival on the two cut treatments had declined to 45 and 27 percent for the areas with and without silvicide, respectively, while on the basal-spray area it was down to 64 percent. Many of the pines needed release—on the cut areas from hardwood sprouts, and on the basal-spray areas from invading herbs and vines and from the expanded crowns of the few surviving hardwoods. Had adequate release been given at the end of the second year, 80 percent of the pines on the basal-spray areas might have survived, as compared with less than 50 percent on the other treatments.

The poor showing of 2, 4, 5-T solution on cut stumps appears to be related to time of application. In tests made during the growing season even more dilute solutions on cut stumps have permanently inhibited sprouting. For applications concurrent with planting, which must be done during the dormant season, 2, 4, 5-T is apparently best applied as a basal spray.

JAMES R. DAVIS

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[1]East Texas Research Center, maintained in cooperation with Stephen F. Austin State College, Nacogdoches, Texas.

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Concentrated or Diluted 2,4,5-T as a Supplement to Girdling?

John J. Stransky

THIS ARTICLE reports on tests installed to determine whether full-strength 2,4,5-T concentrate, applied in a fine spray, would control sprouting and hasten crown kill of hardwoods girdled by ax or machine. An earlier study evaluated the effect of 2,4,5-T on cut stumps (*2*).

A supplementary chemical treatment is needed where vigorously sprouting hardwoods are girdled to release small seedlings, or where, as in east Texas, sprout control is essential to conserve limited moisture (*4,5*). An effective concentrate would reduce the bulk of liquid enough so that the silvicide could be carried and applied by the ax man or operator of the girdling machine, thus eliminating the heavy transportation costs and separate application required with standard dilutions.

Design[1]

Post oak (*Quercus stellata* Wangenh.) and sweetgum (*Liquidambar styraciflua* L.) were chosen as test species because of their prevalence as weed trees on upland pine sites and because both sprout prolifically when girdled. In some areas sweetgum has also shown a tendency to produce enough callus growth to bridge the shallow cut made by the power girdler.

The study was conducted on upland pine-hardwood sites within the Stephen F. Austin Experimental Forest, near Nacogdoches, Texas. Soils are sandy loams overlying clay, and fairly representative of the better Upper Coastal Plain sites in east Texas. Study trees were of two d.b.h. classes, 3.6 to 5.5 inches and 5.6 to 7.5 inches. All had single stems and were judged free of underground connection with their neighbors.

Girdling was either by ax, in which case a standard double-hack girdle was cut, or by the "Little Beaver" power girdler, produced by the Haynes Manufacturing Company Livingston, Texas. This gasoline-powered machine employs a flexible shaft to power a cutting head which produces a smooth, rounded incision about ¾ inch wide. Properly used, it cuts through the bark and well into the sapwood, completely severing the cambium.

The chemical was a commercial preparation of 2,4,5-T, Dow Esteron 245, containing 42.5 percent propylene glycol ether ester by weight (4 pounds acid equivalent per gallon). The dilute solution was made up with Diesel oil, in proportion of 1 to 50, and was painted on the trees with a brush. The concentrate was applied to both ax and machine girdles from a plastic

THE AUTHOR is on the staff of the Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture, East Texas Research Center, maintained in cooperation with the Stephen F. Austin State College, Nacogdoches, Texas.

[1]This study was designed and partially installed by T. A. Harrington.

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squeeze bottle which dispensed a very fine spray. Both dosages were applied in such quantity as to give approximately equal amounts of 2,4,5-T acid to each tree.

The main installations, in April 1955, subjected sweetgums and post oaks to ax and power girdling without chemical, with dilute chemical, and with the concentrate. On each of the two species the six treatments were randomly applied, so that ten trees in each of the two size classes received each treatment. There were three replications of the treatment pattern.

Since other workers (*3, 6*) have found seasonal differences in response to silvicides, parts of the experiment were replicated in January, July, and November 1955. Five sweetgum trees were treated per size-treatment group. Treatments were limited to power girdling with dilute, concentrated, and no chemical. There were three replications each season, as in the main study.

The amount of concentrate or equivalent applied in each treatment is shown in Table 1.

All trees were inspected one and two full growing seasons after being treated. Crown condition, number of sprouts per tree, and length of dominant sprout were criteria for determining treatment effectiveness. This report is based on the second year's data.

TABLE 1.—CONCENTRATION OR EQUIVALENT PER TREATMENT

Season of installation	Species	2,4,5-T concentrate per tree	
		As concentrate	As dilute solution
		Cc	*Cc*
Winter	Sweetgum	1.3[1]	1.0
Spring	Sweetgum and post oak	.9[1]	1.0
Summer	Sweetgum	1.0	.7
Fall	Sweetgum	1.0	1.0

[1]Thinned 50-50 with Diesel oil to facilitate application in low temperature. This slight dilution produced results comparable to the full-strength concentrate and was not distinguished in the analysis.

TABLE 2.—AVERAGE PERCENT OF SWEETGUM AND POST OAK TREES CROWN-KILLED AND COMPLETELY DEAD TWO YEARS AFTER TREATMENT IN APRIL 1955

Treatment	Crown-killed with and without sprouts	Crown-killed without sprouts
	Percent[1]	
No chemical		
Power girdle	75	40
Ax girdle	90	46
Mean	85	42
Concentrated 2,4,5-T		
Power girdle	99	55
Ax girdle	96	57
Mean	99	56
Dilute 2,4,5-T		
Power girdle	99	87
Ax girdle	100	86
Mean	100	87

[1]Mean values weighted by arc sin transformation.

Method of Girdling

Without chemicals, ax girdling killed crowns of 90 percent of treated trees, significantly more than girdling with the machine, which killed 75 percent (Table 2). The difference was greatest in post oak; there tops of all ax-girdled trees died within two years. With chemicals, the two girdling methods were substantially equal, both in top-kill, which consistently exceeded 90 percent, and in percent of completely dead trees, which varied with concentration.

All tops still alive after two years were damaged, and are expected to die eventually except in the few cases where the girdle has been bridged by callus tissue, or where the tree is sustained by root grafts. The difference between the two girdling methods, therefore, is believed to reflect promptness rather than completeness of kill. With both methods sprouting was excessive without chemicals.

Chemicals

All chemical treatments gave a highly significantly better topkill than simple girdling. Both concentrated and dilute 2,4,5-T were effective, killing the crowns of more than 97 percent of the treated trees.

While there was little difference between concentrated and dilute acid in top-kill efficiency, the concentrate proved to be a poor sprout inhibitor. In the April tests, the average percentage of completely dead trees was not high enough for the concentrate to be significantly better than simple girdling; in the seasonal test with sweetgum the complete kill percentages were identical (39 percent) for both of these treatments (Fig. 1). The dilute solution, with a complete kill of 87 percent on both species after spring application, and an average of 56 percent on sweetgum in the seasonal test, was significantly more effective, and provided acceptable sprout control.

Of the trees that remained alive below the girdle, those that received dilute acid applications developed fewest sprouts. Results with the concentrate were only a little better than those from simple girdling. The height of the dominant sprout follows a similar pattern, averaging about 0.8 foot shorter with dilute 2,4,5-T than with the other two treatments.

The superior effectiveness of the dilute solution suggests that the Diesel oil rather than the 2,4,5-T might be the effective agent. Studies by Campbell and Peevy (*1*), however, as well as informal tests in Texas, indicate that Diesel oil alone has little sprout-inhibiting quality. It appears rather that effective quantities of 2,4,5-T are translocated into tissues below the girdle only when diluted. This parallels experience with 2,4,5-T on cut stumps, where large quantities of dilute chemical were most effective (*2*). Apparently the rather viscous concentrate is not readily absorbed into the wood, while the thin Diesel-oil solution penetrates much more readily.

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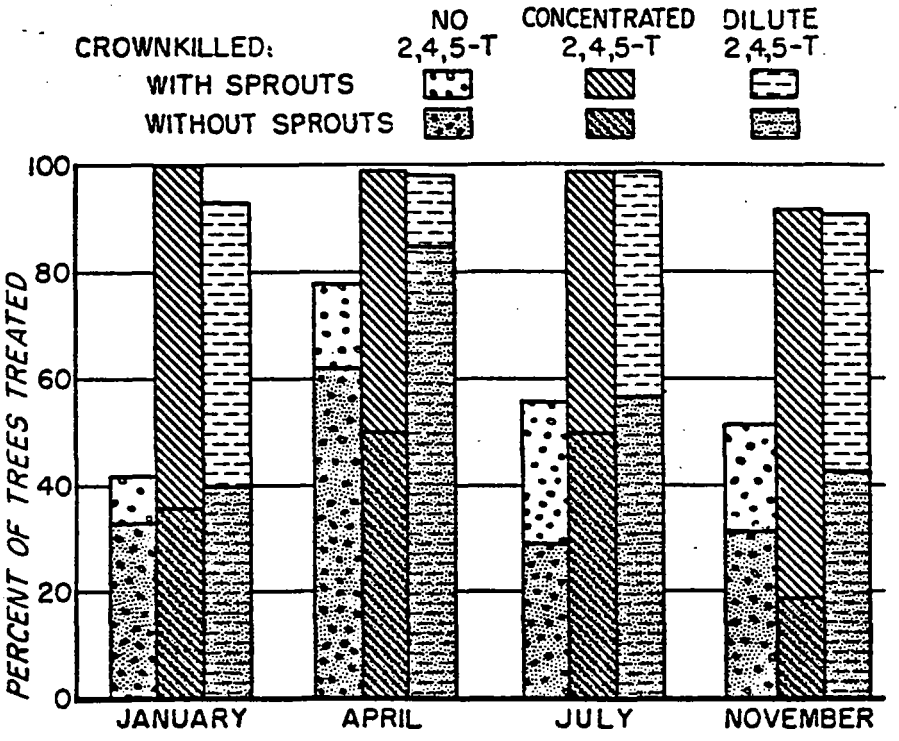


FIG. 1.—Crown kill and sprouting of girdled sweetgums, by season and treatment (average of ax and machine girdling).

Season, Species, Tree Size

In the sweetgum tests, spring treatments were most effective in both top-kill and inhibition of sprouts. Treatments in summer were better than those in fall and winter (Fig. 1). Similarly, on trees not completely dead, sprouts were fewest and smallest after spring treatment. While the differences between seasons were not statistically significant, they are consistent with those reported by other workers (*3, 6*).

Without chemicals about 75 percent of the post oaks sprouted after girdling as compared with about 40 percent of the sweetgums. The difference was not statistically significant, and is inconsistent with considerable experience which rates sweetgum the more difficult species to control.

The tests were confined to a rather narrow range of diameters—3.6 to 7.5 inches d.b.h.—because these size classes have generally sprouted most vigorously after girdling. Within this range, d.b.h. of tree was significant only in the percent of sweetgums top-killed in the seasonal test. Here smaller trees were top-killed more effectively than large ones. Differences were largest where no chemical was applied.

Conclusions

Ax girdling was significantly better than power girdling only in top-kill without chemical treatments. With chemicals there was no difference between ax and power girdling. Top-kill differences reflect promptness rather than eventual completeness of kill, since remaining tops are moribund.

All treatments involving 2,4,5-T resulted in significantly higher top-kill than girdling alone. Concentrated and dilute 2,4,5-T killed crowns equally well; the concentrate, however, proved to be a poor sprout inhibitor, while the dilute solution was very effective.

There was no significant difference between the response of sweetgum and post oak.

Within the limited diameter range of the tests, size of tree did not affect results significantly.

Dilute 2,4,5-T was most effective in reducing sprouting when applied in early spring, least effective in the dormant season.

It is evident that the saving in material costs and transportation of dilutent envisaged in the design of this study cannot be fully achieved by application of undiluted 2,4,5-T. Where promptness of top kill is a primary consideration, application of full-strength 2,4,5-T in cambium girdles may prove useful. Where both prompt kill and inhibition of sprouts are required, the chemical should be applied in dilute form.

Further tests are needed to determine whether some intermediate concentration of 2,4,5-T can be effective without being too bulky for use with a power girdler.

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T-CN- Phenoxyacetic Ac
2,4-dichloro-
K-2372-(100)

THE DOW CHEMICAL COMPANY
BIOCHEMICAL RESEARCH LABORATORY
JOURNAL ABSTRACT

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SUBJECT(S) OR COMPOUND(S): 2,4-D

AUTHOR(S): J. Soó and P. Kertai (Med. Univ. Budapest).

TITLE: Effect of Dichlorophenoxyacetic Acid (DCA) on the Iodine-131 Uptake of the Thyroid.

JOURNAL REFERENCE: Acta Physiol Acad. Sci. Hung. 14, 367-9 (1958) (in English)

ABSTRACT REFERENCE: Chem Abst. 53, 7433 l, April 25 (1959)

ABSTRACT:

Rats were occasionally lose wt. when worki with DCA, its effect on th thyroid was studied. 128 mg. orally to rats in 5 days caused reduced food consumption, reduced activity + signs of systemic poisonin. These rats given 10 μc KI^{131} I.P. + removed thyr 3 hours later — Ave I^{131} uptake was 18% + 8% in controls. Sub. cut inj of 12.5 mg/day resulted in inc uptake to 13.5%. when repeated 2 days + 30.8% after 5 days of treatment. No inc. in thyroid wt.

So what - Acute Tox. VKR.

LABORATORY: ?

TYPE OF ABSTRACT: As above.

BY: VKR.

DATE:

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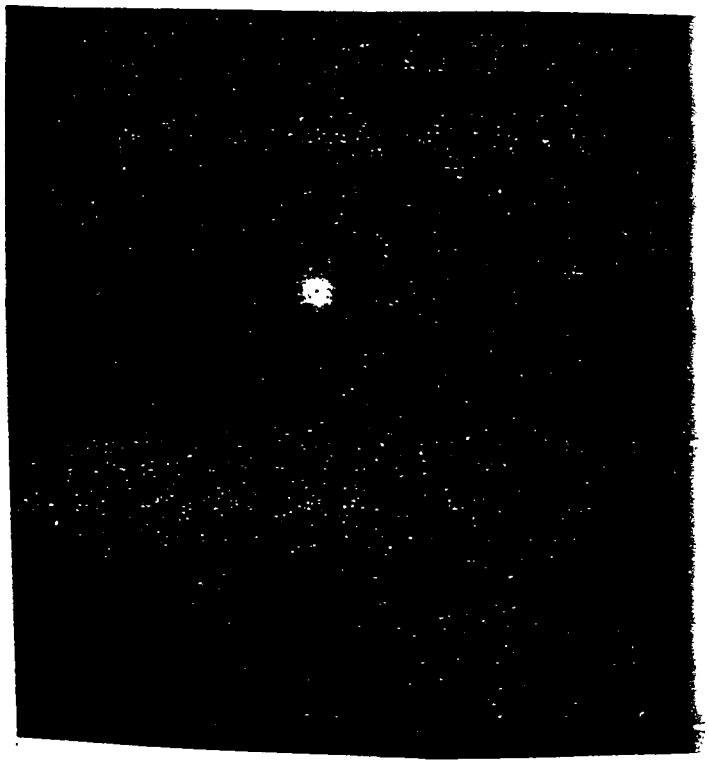
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C. A. Apffel*: The Cytostatic Action of Certain Auxins; Preliminary Review [Action cytostatique de certaines auxines; Compte rendu préliminaire].
Presse Medicale, 67, (6), 207-209 (1959).



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THE CYTOSTATIC ACTION OF CERTAIN AUXINS; PRELIMINARY REVIEW

O. Warburg has postulated that a cancerous cell cannot survive as such if its deficit of respiratory energy is covered by a sufficient augmentation of fermentative glycolysis [1]. *In 1954, I attempted to get a cytostatic effect using monoiodoacetate of soda which reduced the level of fermentative glycolysis without influencing respiratory ferment* [2]. *The result was diametrically opposed to the anticipated effect and the stimulation which it gave sparked my interest in auxins and phytohormones which are antagonistic to iodoacetic acid* [3].

Fundamental principles

Optimal doses of auxins like indoleacetic acid and 2,4-dichlorophenoxyacetic acid can increase oxygen consumption of vegetal tissue from 20 to 60% [4]. This respiration increase accompanies a carbon hydrate depletion. Strong concentrations inhibit growth and respiration in the same way as fermentative glycolysis [5]; but respiration can still be kept elevated at those doses which inhibit growth. Auxin activity is thwarted by reagents of -SH groups such as iodoacetic acid. Thus auxins act through intermediary sulfhydryl enzyme groups, most particularly those dehydrogenases of the Krebs cycle. Interaction with the -SH group of coenzyme A should be foreseen as possible. Auxin action resembles coenzyme action. It participates in most phosphorylation systems and contributes to the aerobic transport of phosphates [6]. In plants, auxins are bound to fraction I vegetable proteins. This fraction has a phosphatase activity and can, along with animal phosphatases, catalyze transphosphorylations [7]. In stimulating concentrations, auxins promote water absorption by vegetable cells, absorption which depends on an oxidative metabolism and very particularly on oxidative phosphorylations [8]. Moreover, these substances provoke in tissues a considerably diminished potassium rate which is elevated in

malign tumors. But their most remarkable property is to influence the decisive work of the nucleic acid content of cells. DNA and RNA rates increase or abate as a function of auxin concentration [9]. Finally, auxins are antagonistic to such cell division factors as adenine and kinetin [1].

Particularly interesting is the action of phytohormones on the tissues of cultured vegetable tumors ("crown galls"). Weak concentrations totally lack a stimulating action [11]. On the other hand, the growth of tumorous sunflower and periwinkle tissues lessens in the presence of weak concentrations of indoleacetic acid, indolebutyric acid and 1-naphthaleneacetic acid. More elevated concentrations (1×10^{-4}) totally inhibit this growth. Such concentrations also stimulate normal sunflower tissue [12]. At 1×10^{-4}, indoleacetic acid stops the wet weight increase of tumorous tissues and tissues "accustomed" to scorzonera [13]. The 2,4-D inhibits tumorous sunflower tissue at concentrations of 1×10^{-13}, and tumorous tobacco tissue from 1×10^{-5} only.

Auxin action in plants increases as they grow more rapidly; young tissues are more susceptible than old ones. In one and the same plant, the less mature and less differentiated cells are the most responsive.

Struckmeyer and his collaborators [14] have histologically observed transformations of tumorous sunflower tissues under the influence of auxins. Weak concentrations have not, on the whole, modified wet weight but have diminished meristem cells per surface unit and have increased scalariform vessels. At strong concentrations, the number of cells of all types greatly diminished and the wet weight fell impressively.

Proof

In spite of these facts, auxins have been neglected in the search for cytostatic substances, no doubt for the following reasons. The mere name "growth substances", implying a growth stimulant, may be one of the causes of their omission. But such designations are deceptive, since auxins can, according to their concentration, also stimulate as well as inhibit growth of vegetable

tissue, and since weak concentrations, again, inhibit tumorous tissue.

Besides, auxins have attracted to themselves the reputation of being carcinogenic. Previously the formation of a callus at the surface level appeared suspect, even though it was really a benign hyperplasia. Gautheret [15] advanced a most serious argument: From a carrot tissue which he had cultivated for many years in a cultured medium increased with 0.1 mg/ℓ indoleacetic acid, Gautheret isolated a cellular strain able to continue to grow and proliferate without any additional indoleacetic acid. This new tissue, which he called "habituated tissue", was friable and translucid, consequently morphologically modified. Grafted onto a healthy plant, it became a true tumor [16]. This transformation of "habituated" tissue, apparently cancerous tissue, was generally attributed to auxin's influence. It is acceptable today to doubt this type of perception. In effect, Sanford and his collaborators, starting from a single mesenchymal cell, successfully isolated at the end of twenty months a cellular strain ("clone") which, in 97% of the cases, instead of forming sarcomas, inoculated the cells of healthy mice [17]. Other authors likewise have observed *in vitro* such a transformation of normal mammal cells into cancerous cells [18 The appearance of malignity lasted from four months up to four years. Goldblatt and Cameron attributed the malign appearance to an anaerobiase. In none of the other cases was it possible to determine the cause of a given carcinogenic agent. In fact, Sanford came to the following conclusion: all cell strains, cultivated in a heterologous medium for a sufficiently long time, finally acquired the property of giving birth to either sarcomas or carcinomas when these cells have inoculated animals of the species from which the culture originated. In other words, every tissue culture is eventually carcinogenic.

Two other facts have provoked the thought that phytohormones can be carcinogenic: a) the *Bacterium tumefaciens*, agent of vegetable tumors, produces indoleacetic acid in an artificial medium; b) vegetable tumors contain more auxin than corresponding healthy

tissue. However, indoleacetic acid production by *B. tumefaciens* reaches at most 125 micrograms per liter [19], when 10-30 million micrograms is necessary to artificially provoke a gall [20]. Other bacteria also produce auxins in an artificial medium; but they never instigate the formation of tumors [21]. The high auxin rate verified in vegetable tumors is due to the fact that because of their incomplete oxidations, they consist of more free auxin and less bound auxin in protein fractions. The auxin-protein connection depends in effect on normal oxidations and the presence of adenosinetriphosphate [22].

Another reason for eliminating auxins from the list of actual cytostatics is the fact that these are substances whose action is strictly limited to vegetable metabolism. However, past and present publications report on work proving their influence in animal metabolism. We recall the discovery of the regular presence of auxin *a* and indoleacetic acid in human urine [23] which was attributed to the action of intestinal bacteria. In 1949, Raoul and Marnay verified a growth stimulation in young rats under the effect of weak doses of indoleacetic acid [24]. Zambotti and De Bernard threw into relief indoleacetic acid's influence on respiration and glycolysis in animal cells [25]. Finally the works of Louis have shown an impressive parallel between indoleacetic acid's effect on the cultured tissues of vegetables as well as animals. In anaerobic conditions, weak concentrations have been stimulants and strong concentrations have been inhibitors to cultured cardiac cells of chicken embryos. In aerobiosis, only inhibition by very strong doses was observed. Indoleacetic acid distinctly works on nuclear division; increasing concentrations severely diminish the number of mitoses [26].

Thus, I have led up to the search for whether auxins, whose cytostatic effect on "crown galls" has previously been pointed out, can as well inhibit animal and human tumors.

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Toxicity

Given the massive use of auxins in agriculture, their toxicity has been tested several times. Six grams of 2,4-dichlorophenoxy-acetic acid was administered to cows for three and a half months without provoking the least symptoms of intoxication. A research worker has taken 0.5 grams of the same substance for twenty-one days without disturbance.

In spite of this, I verified one more instance of 2,4-D toxicity, this time in the form of ethyl ester, among rabbits. At first, a dose of 0.01 g/kg of weight was injected intramuscularly. After five days, the dose was increased to 0.02 and, after another five days, to 0.05 g/kg. The experiment was stopped at the twentieth day. The appetite and vivacity of the animals were not at all impaired as the doses were raised. As a continuation, I parenterally administered auxin to animals and humans in average daily doses of 3 to 4 mg/kg for very long periods, without ever observing toxic phenomena or undesirable reactions. A patient received, for one hundred forty days, an ethyl ester of 2,4-D, then an isopropyl ester of 2,4,5-trichlorophenoxyacetic acid in daily intramuscular doses of 0.10 to 0.30 grams without presenting symptoms of intoxication. These products were employed in an olive oil solution. Intragluteal injections caused neither burns nor aches and were easily reabsorbed. During the course of a year, the hundreds of injections never provoked an abscess.

Concerning administration *per os*, it should be noted that daily quantities of 0.30 g and more (divided into doses of 0.10 g) soon released a laxative effect. Other subjects, particularly those given 2,4-D and 2,4,5-T esters, experienced a disagreeable gout.

The fact ought to be emphasized that hematopoietic organs and the blood formula was not influenced. The rates of cholesterol, urea, and glucose in blood were never augmented. Only a slight hypoglycemia was occasionally verified. Arterial pressure was at no time raised during the treatment. We are dealing with a group

of very weakly toxic substances, not having, in opposition to the antimetabolites and nitrogen mustard derivatives, any effect on hematopoiesis.

After not having ever exceeded daily doses of 0.45 g from October 1956 until October 1957, I decided to use daily quantities of 1.25 to 2.50 g (once at 1.25 to 1.50 or twice at 1.0 to 1.25 g intramuscularly). Even at these very elevated doses, the injected substance was painless and easily reabsorbed; on the other hand, certain disturbances soon appeared. They consisted of a lassitude, particularly in the legs, a heightened thirst, vertigo, and, in two cases, a slight confusion. The last two symptoms did not appear at the most elevated doses. The patients exhibited then a certain inebriety. Twice I noted diarrhea, and once, vomiting. A few days after the interruption of treatment, the toxic symptoms disappeared completely. As a notable secondary effect during treatment with these elevated doses, arterial pressure markedly diminished.

Drill has pointed out among mammals the following toxic symptoms: weakness, diarrhea, weight loss, and, in certain species, a curious state of a myotonic response to quinine [27].

Preliminary analyses

The initial analyses were attempted with indoleacetic acid and the sodium salt of 2,4-dichlorophenoxyacetic acid. The first was mixed with equimolecular quantities of piperazine, rendered thus hydrosoluble, and injected intravenously in a 1 to 2% solution at a rate of 0.02 to 0.10 g/day. The second, hydrosoluble naturally, was administered in the same fashion in a dose of 0.10 g/day. Five cases (a lymphogranulomatosis, i.e., Hodgkin's disease, a metastatic carcinoma mastitoides, a lymphosarcoma, a multiple metastatic stomach cancer, and an adenocarcinoma of the prostate with postoperatory metastases) were thus treated for twenty-five days without either amelioration or aggravation. Neither of the two substances provoked the appearance of toxic symptoms. The injections were painless, non-hardening, and did not release secondary reactions

These analyses seem to confirm the inactivity of auxins in humans in contradiction to the qualified observations on the cultured tissues of animal origin. One can ask if, at a very elevated human blood pH (pH of human blood: 7.3 to 7.4; pH of vegetable tissues::5.5 to 6.0), auxins have not been dissociated to the point of losing their activity. Effectively, Audus has been able to verify that a weak dissociation of 2,4-D is linked to an elevated toxicity for plants. Already at a pH of 3.28, 50% of the molecules were dissociated. Audus admits that 2,4-D works uniquely as a non-dissociative molecule [28]. Among the derivatives of indoleacetic acid and phenoxyacetic acid, the alkaline salts and the amides are ionized in the same style as the acids; only the ester and nitrile molecules and the corresponding aldehydes continue to be non-dissociative. Analysis is thus followed up for these latter categories, principally with the esters.

Analyses with non-ionizable auxins

Derivatives employed:

From October 15, 1956 to October 11, 1957, auxins were administered in daily doses of less than 0.45 g. I successively employed the ethyl ester of 2,4-dichlorophenoxyacetic acid, and the isopropyl ester of 2,4,5-trichlorophenoxyacetic acid. The first exists on the market in the form of a brown liquid, soluble in any proportion of vegetable oil. Even at the strongest concentrations (50%), the solutions stay clear and resistant to sterilization. The second appears in the form of a light brown crystal composed of a compact mass that ought to be melted at 80° to 90° to be able to dissolve in olive oil. Mixed in cold oil, the substance immediately recrystallizes; but after reheating (sterilization), the solution stays stable, at least up to 15%.

By October 1957, the impression became clear that a daily dose of 0.10 to 0.20 g of these esters could not bring about in humans conditions which corresponded to a concentration of 1×10^{-7} to 1×10^{-3}, because such concentrations were the minimum rate necessary to obtain total inhibition in a vegetable tumor culture.

One must in reality administer doses near 1.0 g at least and, since it is important that the rate in the interior level be maintained above 1 mg % for the whole day, *per diem* quantities of 1.50 to 2.50 appear appropriate. However, the administration of such quantities is not possible with 25 to 50% solutions or with isopropyl esters of 2,4,5-T given its limited solubility. I am obliged thus to return to 2,4-D ethyl ester; but I have run up against the toxic troubles I indicated before. I am forced then to find more active derivatives that allow administration of smaller quantities. The ethyl and methyl esters of 2,4,5-trichlorophenoxyacetic acid fulfill these conditions. Phytobiological tests fixed at first an activity five times greater than that of 2,4-D ethyl ester. These white and compact needle crystals do not have a limited solubility in olive oil. Instead of the projected 30% solution, a 20% solution was prepared with the addition of 5% acetonitrile. The substance recrystallized however and it had to be reheated in a boiler to redissolve it before each injection. Above 30° the solution was stable. None of the experimental data, phytobiological or otherwise, has been published concerning the 2,4,5-T methyl ester. One knows nevertheless that the esters of phytohormones inhibited germination and budding more as the radical alcohols of these esters allowed the number of carbon atoms more variance. The white 2,4,5-T crystals of methyl ester are again less soluble than those of ethyl ester. The recrystallization of a prepared 10% solution required reheating in a boiler before injection. The fear that instigated the use of the methyl ester was now confirmed. The methyl 2,4,5-trichlorophenoxyacetate could be administered parentarally in doses of 0.85 g per day for six weeks without provoking the least apparition of trouble.

The effects of the two primary derivatives employed (ethyl 2,4-dichlorophenoxyacetate and isopropyl 2,4,5-trichlorophenoxyacetate) were *grosso modo* the same and the second did not exceed by a bit the first in efficiency. The ethyl and methyl esters of 2,4,5-trichlorophenoxyacetic acid proved to have a cytostatic activity superior to that of the isopropyl ester. This precedence

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inspired confidence in methyl ester whose tolerance was perfect. Successfully ameliorating its solubility will allow highly elevated doses to be given.

Clinical experience:

Of the 41 cases treated, 5 were animals.

The auxins used had a particularly marked effect on benign tumors. In four cases of fibroadenoma of the prostrate, the troubles (retention, dysuria, pains, and stuttering and precipitant urination) disappeared during the first week. At the end of ten to fifteen days, hypertrophy had retroceeded in such a manner that the treatment could stop on the fifteenth day. Efficacy was also remarkable on a recently appeared diffuse adenoma of the right lobe of the thyroid. After fifteen injections of 0.15 g of 2,4,5-T isopropyl ester, a goiter, as large as a prune, disappeared. A fibromyoma of the uterus retroceeded, during twenty days of treatment, from the size of a grapefruit to that of a detectable pain curve.

On cancerous tumors of reduced malignity and without detectable metastases, efficacy was equally remarkable; there was always amelioration and, in three cases, recovery (one facial fibroepithelioma in a donkey and two epitheliomas of the type *ulcus rodens* in a dog and a cat).

In cases of average malignity, there was a clinical recovery (tumor at the point of cholecystic parting in a 55 year old woman with enormous metastases of the mesenteric ganglia), ameliorations, momentary halts, or a slackening of the process occurred. The cases of great malignity (for example pulmonary cancer of the Pancoast type) or preagonic malignity reacted hardly or not at all.

It is noted that at doses less than 0.45 g, the esters of 2,4-D and 2,4,5-T were perfectly well supported by infants. A seven year old boy, afflicted with a malign lymphogranulomatosis, received a daily dose of 0.10 g for three months without any undesirable secondary reaction.

Among the treated cases were mammary adenocarcinomas, bronchogenic carcinomas, rectal cancers, epitheliomas, a lymphoma, a lymphosarcoma with multiple tumors, etc., but no leukemia.

Radiotherapy a little (less than three weeks) prior to auxin treatment increased tumor sensitivity in these lymphosarcomas. A remarkable effect was observed in a man affected with a bronchogenic carcinoma who, a few days before treatment with 2,4-D ethyl ester, had received 1500 r in six sessions. A fifty-eight year old woman, suffering from an inoperable cancer of the uterus, reacted in such an extremely favorable manner at that time to 2,4-D ester treatment that she then received a total dose of 11,480 r. After 48 injections starting on December 24, 1956, she remains clinically healed.

Radiotherapy and treatment with esters of auxins, applied simultaneously, seem to have a reciprocal potential. A proof was furnished by a 56 year old woman afflicted with a lymphosarcoma with multiple tumors. A single treatment of 2,4-D ethyl ester gave an amelioration; but the patient had developed a resistance and did not react to a second series. On April 4, 1957, treatment was begun again, this time with 2,4,5-T isopropyl ester at a rate of 0.10 g/day intramuscularly, simultaneous with X-ray radiation. Although the patient received in all, as of May 12, 1957, only 2100 r (fifteen sessions of 125-150 r under 175 kw.with 1 mm Cu and 1 mm Al, distributed across four areas, two cervical and two axillary), the gangliary tumors nearly totally retroceded. This result, for a second radiotherapy and considering the given dosage, was unforseen. In another case, also treated simultaneously with X-rays and a 2,4,5-T isopropyl ester, a total dose of 2250 r spread over twenty-two days sufficed to completely dissipate Hodgkin's tumors. This also was a question of radiotherapy repeated for the second time. In a third case, where the efficacy of esters of auxins seemed to have been exhausted, a simultaneous radiotherapy resulted in new and palpable progress. In an osteosarcoma of the thigh, on the other hand, simultaneous radiation was a disastrous influence and had to be stopped.

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The efficacy of esters of auxins was at a maximum for the first two weeks, then began to decline; but stayed still notable for one of two weeks. After a month, there was hardly any progress at all. With 2,4,5-T isopropyl ester, efficacy diminished perhaps a little faster. It seems thus to develop a certain resistance.

The efficacy of esters of auxins regularly increased through simultaneous administration of riboflavin *per os* and intravenously.

Note that the auxins employed were contaminated by polychlorophenol. These latter considerably augmented the action of auxin-oxidases by which furthermore they induced or amplified formation [29] It is possible that these polychlorophenols are in part responsible for the resistance provoked above. As with 2,4-dinitrophenol, they disconnect respiration, properly called oxidative phosphorylations, and prevent the formation of ATP. However, the commercial esters of auxins of which I availed myself continually bring to mind isomers such as 2,6-D and 2,4,6-T. These isomers inhibit the action of active auxins. Also, in the clinical analyses which have been attempted in a Parisian hospital, we exerted ourselves to utilize substances carefully purified by recrystallization in the appropriate solvents. We further hope to be able to test, on an index of raised activity, other auxins which I was not able to prepare in sufficient quantities.

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Discussion

In 1953, Silberberger and Skoog [9] proved that auxins like indoleacetic acid work in a direct fashion on the amount of nucleic acids in vegetable tissues and that growth depends to a large extent on this relation. The amount of ribonucleic acid and deoxyribonucleic acid in a vegetable tissue cultivated *in vitro* begins to increase at the moment when increasing quantities of indoleacetic acid are added to the culture medium. For deoxyribonucleic acid, this increase is optimal at 0.014 mg/ℓ; for ribonucleic acid, it is only optimal at 3.3 mg/ℓ. Exceeding the optimal doses of indoleacetic acid diminishes the amount of nucleic acids. At

90 mg/ℓ, it ought to be lower than that of the controls and finish by reaching -50% and less towards 270 mg/ℓ. Increase and decrease of this amount parallels the fluctuations in wet weight. We recall here Struckmeyer's observations [14] on the tissue cultures of vegetable tumors. He remarked on a considerable drop in the wet weight under the influence of strong concentrations of auxins. We also recall here that 2,4-D ethyl ester is more active than indoleacetic acid and that tumorous tissue is only sensitive to the inhibitory effect of phytohormones. We can thus suppose that at elevated concentrations of certain auxins, the tumor's amount of ribo- and especially deoxyribonucleic acids can be reduced. This is particularly important if one bears in mind the decisive role that ribo- and especially deoxyribonucleic acids play in the division of nuclei and cells. From 1953 to 1956, Miller [30], starting with a preparation of deoxyribonucleic acid from herring sperm, successfully isolated a highly cytokinetic substance, whose presence was impartially verified in analogous preparations from the thymus of a calf and in yeast extract. This factor, which he called kinetin, was capable of releasing cellular division in a concentration of one µg/ℓ. It turned out to be a puric derivative, 6-furfurylaminopurine. Fresh preparations of deoxyribonucleic acid did not exhibit kinetin action; this only appeared after abandoning the preparation for months or after a sojourn of sixty minutes in an autoclave at 125°. The kinetin seems to be a product of deoxyribonucleic acid disintegration and it is correct to suppose that its presence in tissues is strictly dependent on the amount of deoxyribonucleic acid. It thus seems justified to propose the hypothesis according to which the cytostatic action of an auxin is explained by the rarefaction of cell division factors such as kinetin. From the work of Skoog and his school [31], it follows that, of the two elements of vegetable growth, elongation and cellular division, the first depends on having all of the auxin concentration; the second, on the factors such as kinetin or an integral part of this last, adenine. A modification between the proportions of adenine and auxin favoring the first expresses

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itself by an increased DNA content; a modification favoring auxin finds its expression in an elevation of the RNA content. This leads us to propose the following equation:

Adenine (kinetin)/auxin = DNA/RNA = division/elongation.

Skoog and Tsui have shown that elevated adenine concentrations and weak auxin concentrations favor budding and that on the contrary budding was inhibited by elevated auxin and weak adenine concentrations [32]. This antagonism between the factors of cellular division such as adenine (which can be potentialized by pyrimidines like guanine, thymine, and cytosine) and kinetin on the one hand, and the factors of elongation such as indoleacetic acid on the other hand, is of a very peculiar nature. In fact, kinetin is only active in the presence of traces of auxin [33]; inversely, it seems that auxins need the presence of adenine to have an effect on cellular elongation [34]. Still, a great preponderance of the one can stifle the characteristic effect of the other [35].

It is perhaps daring to transpose into the domain of animal biology facts discovered in that of phytobiology; for the moment however, these latter alone can help us understand the effect of auxins on tumors in humans and animals.

Summary

In weak concentrations, auxins accelerate the catabolism of carbon hydrates, activate oxidative phosphylorations, increase oxygen consumption in tissues and stimulate growth. In elevated concentrations, they complete the depletion of carbon hydrates, obstruct anaerobic glucolysis, reduce the rate of potassium in tissues, and inhibit growth. Auxins regulate the rate of nucleic acids and are antagonistic to factors of cellular division such as kinetin or 6-furfurylaminopurine. Their inhibitory action on growth is remarkably selective. It is most evident for certain vegetable species than for others and, in all cases, stronger as the tissue in which it operates is younger and less differentiated.

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The utilization of auxins as synthetic herbicides is based on these properties. Tissues of vegetable tumors are more sensitive to auxin's inhibitory action than corresponding normal tissues.

Auxins operate, although to a lesser degree, on animal metabolism. The author has undertaken to utilize certain auxins for the selective inhibition of benign and malignant tumors in humans and animals. Auxins in ionizable form prove to be inactive. On the contrary, non-dissociable derivatives (esters of alcohols) manifest considerable cytostatic action. The ethyl ester of 2,4-dichlorophenoxyacetic acid, the isopropyl ester of 2,4,5-trichlorophenoxyacetic acid, and especially the methyl ester of the same acid are particularly active. The anti-tumor effect of these auxins is reinforced by riboflavin. Radiotherapy concomitant or slightly preceding (less than three weeks) treatment with auxins considerably reinforces the cytostatic action of this last, even when the dose of rays is subliminary [sic]. The cited esters of auxins have a very slight toxicity and never depress hematopoeisis.

Their hyperglycemic effect was confirmed. They were preferably administered in an oily solution by intramuscular injections of 0.50 to 1.0 g/day. Rests of four days can be intercalated between successive series of fifteen days.

The esters of auxins utilized must be purified of any contamination by the corresponding polychlorophenols and neighboring isomers, whether inactive or antagonistic.

Acknowledgments

Among the treated cases, there were five animals. I direct my thanks to Dr. E. C. Preston (The People's Dispensary for Sick Animals, Tangiers) for having permitted the treatment of them. I equally thank him for the efficient help and enlightened counsel that he so often squandered on me. However, I have to express my gratitude to Messieurs the Pr. H. Veldstra and H. Linser at the same time as to the Rhône Poulenc Laboratories, and Phili s-Roxane for the substances which they freely consented to put at my disposal.

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12 5095 Biochemical Research Laboratory

The Dow Chemical Company

RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON 2,4-DICHLOROPHENOXY ACETIC ACID ACIDIFIED WITH CHEMICALLY PURE HCl AND WITH HCl RECOVERED FROM THE PENTACHLORO-PHENOL PLANT

File T23.14-11-11
K No. 2378-24-45
Chg. 1270-26
Sub. by: L. G. White, Jr.
Ref. 5-AW-39-A; 5-AW-45
Rept. by: K. J. Olson

Signed K. J. Olson Checked J. Lyon

Date Feb. 5, 1960 Date 2-5-60

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PROBLEM

Two samples of 2,4-D were submitted on October 12, 1959, for toxicological comparison insofar as skin irritation is concerned. Sample 5-AW-39-A was acidified with chemically pure HCl while sample 5-AW-45 was acidified with HCl recovered from the Pentachlorophenol Plant. The latter sample was suspected of being more irritating to the skin in view of the fact that it might contain residual phenols. A comparison of the two materials was requested.

CONCLUSIONS

The subject sample acidified with HCl from the Pentachlorophenol Plant is no more irritating to the skin than that acidified with chemically pure HCl. Both materials were applied to the intact rabbit belly in both moist and dry forms. In each cases, single prolonged exposure would be expected to produce some reddening in human skin and repeated prolonged contacts would likely cause appreciable irritation and possibly a mild burn. Prolonged contact with either material should be avoided.

For information regarding health hazards and precautions for safe handling of this material, the reader is referred to the current Industrial Hygiene Data Sheet.

(Continued)

DOW 0233957

CONCLUSIONS (CONTINUED)

These conclusions are based upon range finding toxicological tests and are limited to precautions for industrial handling of the material. Development of specific uses will require consideration of the health problems presented and of the need for further toxicological studies.

SUMMARY OF RANGE FINDING TOXICOLOGICAL DATA

Skin Contact - Rabbit 2,4-D sample acidified with CP HCl (5-[illegible]-[illegible])

Material	Condition of Skin	No. of Appl.	Site	Response-Remarks
Undiluted (Wet)	Intact	10	Belly	Sl. hyperemia throughout experiment - skin normal in 21 days.
Undiluted (Dry)	Intact	4	Belly	Sl. hyperemia throughout experiment - sl. to mod. necrosis after 3rd and 4th appl. - skin essentially normal in 21 days.

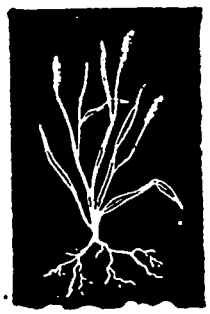
2,4-D sample acidified with HCl from Penta Plant (5-[illegible]-45)

Undiluted (Wet)	Intact	10	Belly	Sl. hyperemia and slight to mod. edema.
Undiluted (Dry)	Intact	10	Belly	Sl. hyperemia and edema throughout the experiment - sl. necrosis after 4th appl. - skin essentially normal in 21 days.

Skin Absorption

There is no indication, from the skin irritation tests conducted, that these materials are absorbed through the skin in toxic amounts.

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Weeds

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BRIEF PAPERS

Iso-octyl Ester of 2,4,5–T in Hardwood Control

CARTER B. GIBBS[1]

DAVIS and Duke (2) in 1955 reported successful use of a 36 lb ahg solution of the propylene glycol butyl ether ester of 2,4,5–T applied by means of a tree injector.[2] This tool is essentially a modification of the Cornell tool described by Cope and Spaeth (3) in 1931, and subsequently tested with ammonium sulfamate (1, 5) and 2,4,5–T (4, 6, 7).

Since the iso-octyl ester of 2,4,5–T is generally available at lower cost than the previously tested propylene glycol butyl ether ester, a study to assess its effectiveness was installed in March 1957 by the Nacogdoches Research Center in cooperation with the Sam Houston National Forest. Each of four concentrations of the chemical, 40, 20, 13.3, and 8 lb ahg in diesel oil, was applied to 30 sweetgums (*Liquidambar styraciflua* L.) and 30 oaks (*Quercus stellata* Wangenh., *Q. marilandica* Muenchh., and *Q. falcata* Michx.). Trees were of two diameter classes, 0.6–3.5 inches and 3.6–6.5 inches. A total of 480 trees were thus injected—all in circumbasal wounds, one per inch of estimated tree diameter.

Since an aim of the study was to examine the effectiveness of various concentrations under field conditions, no special measures were taken to assure exactly uniform applications of the silvicide. Each treated tree was inspected for crown condition and sprouting in May and October 1957 and in November 1958.

At the first inspection, three months after treatment, crown damage was visible only on trees treated with the higher concentrations. By the end of the first growing season the most effective treatment, 40 lb ahg, had top-killed 100 percent of the small trees and 93 percent of the large trees. Top-kill was slower on sweetgum than on oaks. No treated trees sprouted during the first growing season.

Results at the end of the second growing season are shown in Table 1 as percents corresponding to the mean angle of equal information (arcsin $\sqrt{\%}$ transformation). The difference between 100 percent top-kill for 40 lb ahg and 75 percent for 20 lb ahg was highly significant. The 64 percent top-kill with 13.3 lb ahg was significantly more effective than 33 percent with the 8 lb ahg. Differences in complete kill were similarly significant. Differences in both top-kill and complete kill between oaks and sweetgum were significant, an indication that sweetgum was harder to kill than the oak species tested.

There were no significant differences in either top-kill or complete

[1]Nacogdoches Research Center, maintained at Nacogdoches, Texas, cooperatively by Stephen F. Austin State College and Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture.

[2]Manufactured by Reuel Little, Madill, Oklahoma.

Table 1. Top-kill and c
inje

Concentration (lb ahg)	Trees	
	Oak	S
40	100	
20	92	
13.3	69	
8	39	

kill between the two tree size
trees sprouting (including t
greater for the smaller size

The effectiveness of iso-oct
oaks and gums in east Texa
ester of 2,4,5–T can be bas
Where only oaks are to be c
of the iso-octyl ester may be s
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Table 1. Top-kill and complete kill two growing seasons after injection with 2,4,5–T.

Concentration (lb ahg)	Trees top-killed, pct			Trees killed, no sprouts, pct		
	Oak	Sweetgum	Both	Oak	Swertgum	Both
40	100	100	100	100	100	100
20	92	54	75	82	50	67
13.3	69	55	64	64	54	59
8	39	27	33	35	23	29

kill between the two tree size-classes, though the total percentage of trees sprouting (including those with live tops) was significantly greater for the smaller size class.

The effectiveness of iso-octyl ester of 2,4,5–T when injected into oaks and gums in east Texas means that choice of a low-volatile ester of 2,4,5–T can be based on relative costs of the chemical. Where only oaks are to be controlled, the 20 lb ahg concentration of the iso-octyl ester may be satisfactory. Where sweetgum or other species with similar resistance to silvicides are to be treated, a higher concentration will be required.

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anomalies in the electroencephalogram (EEG), consisting of bilateral synchronous theta-wave activity and occasional bilateral synchronous spike and wave complexes, believed to be associated with brain stem injury. These specific disturbances in the EEG may persist for some time after apparent clinical recovery, but they almost always disappear spontaneously after discontinuation of exposure. Systematic survey of a large group of exposed workers may occur without preceding or subsequent convulsions and even without any clinical symptoms or signs. Periodical electroencephalographic examination is a valuable method for the detection of early subclinical intoxication. Moreover, if suspect symptoms suddenly appear during the interval between periodic examinations, particularly if myoclonia occurs, an additional EEG should immediately be taken. Appearance in the EEG of the above-mentioned specific disturbances is an indication for removal of the worker from further exposure. Clinical recovery after convulsions has always been complete. With adequate precautions it is possible to prevent intoxications among workers who have daily contact with the insecticides over a period of years.

-- Cond. from authors' summary

354 Nervous System Effects of a Chemical Herbicide. I. Desi, et al. Arch. Environmental Health 4, 95-102 (Jan. 1962).

After the parenteral administration of 2,4-D a reversible inhibition of cerebral electrical activity was observed in the acute experiments, and in chronic experiments the same was present to a gradually increasing degree. Toxic electroencephalogram signs were developing. According to conditioned-reflex experiments, the higher nervous activity suffered severe damage. The changes are probably produced by the 2,4-D molecule itself and not by some of its degeneration products. The point of attack seems to be the reticular formation. The changes manifest themselves as early as 24 hours after exposure. The changes found in animal experiments suggest the need of caution in the use of 2,4-D. Increased protection and special neurological examination of workmen in contact with 2,4-D is necessary.

-- Authors' summary

INDUSTRIAL DUSTS

355 Maple-Bark Disease. Pneumonitis Due to Coniosporium Corticale. D. A. Emanuel, B. R. Lawton and F. J. Wenzel. New Engl. J. Med. 266, 333-337 (Feb. 15, 1962).

The clinical impression that some types of pneumonitis may be caused by individual hypersensitivity to molds or their products has received increasing attention in recent years. The exact role of molds, dust and individual susceptibility, however, is not fully understood. "Farmer's lung" is thought to be caused by a reaction of this type, but bagassosis and "mushroom-grower's disease" may also be related to such a phenomenon. The authors present a case of pneumonitis due to Coniosporium corticale. The spores were demonstrated microscopically from tissue obtained by lung biopsy, and cultures yielded a fungus identical to the mycelial phase of C. corticale. To the knowledge of the authors this is the first case since Towey and his co-workers (J.Am.Med.Assn. 99, 453-459 (1932)) reported their 35 cases in 1932, and the first demonstration of a diffuse type of interstitial pneumonitis with granuloma formation due to a reaction to a specific fungus spore.

356 Intradermal Sensitivity Testing in Man with a Purified Vaccine for Q Fever. D. B. Lackman, et al. Am. J. Public Health 52, 87-93 (Jan. 1962).

A standard product for use in a quantitative intradermal test to determine sensitivity against C. burnetii has been described. The importance of utilizing organisms in Phase I for preparing vaccines has been emphasized. Recommended modifications in the intradermal test include use of 0.02 complement-fixing unit as the skin-test dose and utilization of induration as the most reliable indicator of sensitivity. Finally, the authors suggest wider use of the intradermal test as a pre-vaccination procedure and in epidemiological studies to determine the prevalence of infection of man by C. burnetii.

-- Authors' summary

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Some Effects of 2,4-Dichlorophenoxyacetic Acid on Thyroid Function in the Rat: Effects on Iodine Accumulation[1]

WARNER H. FLORSHEIM AND SHIRLEY M. VELCOFF

Radioisotope Service, U. S. Veterans Hospital, Long Beach, California, and University of California Medical School, Los Angeles, California

ABSTRACT. 2,4-Dichlorophenoxyacetic acid, a compound widely used as a weedicide, markedly increases I^{131} uptake by rat thyroid *in vivo*. Measurements of a number of thyroid function criteria, including thyroid cell height, rate of discharge of I^{131} from the gland, and serum and pituitary TSH levels, indicate that this effect does not involve the pituitary thyrotrophic hormone but represents a direct effect on the peripheral iodide pool. This was confirmed by isotope equilibrium analysis of the serum iodide level. The effect can be demonstrated only in rats in which the thyroid functions normally, and does not occur in hypophysectomized or iodine-depleted animals.

THE STUDY of the action of drugs upon thyroid function has been a fruitful method of elucidating the details of thyroid metabolism. Several years ago, Sós and Kertai (1), in the course of a study of tyrosine antagonists, noted that 2,4-dichlorophenoxyacetic acid (2,4-D), a commonly used weedicide, had some rather unusual effects upon thyroid function in the rat. They showed that administration of the drug in nontoxic doses for several days led to a marked increase in the thyroidal radioiodine uptake without any effect upon thyroid weight or the fraction of radioiodine that becomes protein-bound. We decided to investigate further the mechanisms underlying these effects of 2,4-D on thyroid function.

Materials and Methods

The 2,4-D used was Eastman "white label" material purified by Datta's (2) method. It was dissolved in 0.15 M sodium bicarbonate solution and administered by daily sc injection. Male Sprague-Dawley rats were housed in a room kept at 24 C and fed Wayne Lab Blox (iodine content: 2.0 $\mu g/g$) and tap water. Thyroid function studies were carried out as described previously (3). Iodine was determined by a modification of Bodansky's wet ashing technique (4). McKenzie's mouse assay (5) was used to assay thyrotropin in rat pituitaries and in concentrates of pooled rat serum prepared by Bates's percolation method (6). Analysis of thyroidal iodinated amino acids was carried out by the ion exchange method of Galton and Pitt-Rivers (7) after digestion with

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[1] Presented at the 4th International Goitre Conference, London, England, July, 1960.

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TABLE 1. Effects of 2,4-D on thyroid function parameters

Test	Rats per group	Controls	2,4-D-treated	Significance of difference (P)
24-hr I^{131} Uptake	9	4.89 ±0.30%*	6.40 ±0.23%	.001
Serum PBI	10	3.2 ±0.1 μg/100 ml	2.7 ±0.1 μg/100 ml	.002
Thyroid Weight	9	9.41 ±0.44 mg	9.52 ±0.33 mg	—
Thyroidal Total Iodine	9	78 ±3 mg/100 g	79 ±3 mg/100 g	—
24-hr Conversion Ratio	6	46 ±3%	44 ±3%	—
T:S	6	31 ±2	48 ±5	.02
Thyroid Cell Height	6	13.0 ±0.8 μ	12.6 ±0.2 μ	—
$T\frac{1}{2}$ of Thyroidal I^{131}	6	5.4 ±0.3 days	5.3 ±0.3 days	—

* Standard error of the mean.

pancreatin (California Corporation for Biochemical Research) in pH 8.2 borate buffer. Only the fractions that could be eluted with aqueous acetic acid were analyzed.

Serum iodide levels were measured by the isotope equilibrium technique as described by Simon and Morel (8). Rats were kept on iodine-free water and the Remington diet (Nutritional Biochemicals Corp.), containing only 16 μg iodine/kg, for 26 days. During the entire treatment period, they received by daily sc injection 1.0 cc of a solution containing, on the first day of the experiment, 0.8 μc I^{131} and a known quantity (either 5 or 10 μg) of stable iodide. During the last 7 days of the regimen, half the rats received 80 mg/kg body weight of 2,4-D, while the rest received the bicarbonate vehicle only. After autopsy, 3 cc of serum from each rat was treated with 5 vol of 10% trichloroacetic acid, the TCA precipitate was washed once with TCA, and its radioactivity was determined in a well-type scintillation counter. To the combined TCA supernates, 5 mg of carrier iodide was added and the iodide was precipitated with an excess of palladium chloride. The palladium iodide precipitate was washed with water and n-butanol and then dissolved in concentrated ammonium hydroxide and quantitatively transferred to planchettes for counting of the contained radioactivity with a gas-flow Geiger counter. The conversion factors for calculating iodide content from the radioactivity data were derived by assaying aliquots of the injection solution for radioiodine and iodine content. Recovery studies showed that iodide recovery was quantitative and that any traces of thyroxine carried down with the palladium iodide precipitate were eliminated readily by washing the precipitate once with n-butanol before planchetting.

Results

We first established a dose of 2,4-D that would consistently duplicate the findings of Sós and Kertai (1). At 80 mg/kg per day, the animals continued to gain weight almost normally, and there was no effect upon thyroid, pituitary, adrenal or testicular weight even when 2,4-D administration was continued for several weeks. At 100 mg/kg, both thyroid and body weight decreased, and at higher doses paralysis occurred. Table 1 summarizes some of the effects upon thyroid function parameters. The effect of the serum PBI, which appears to be caused by changes in serum and tissue binding of thyroxine, will be discussed fully in a subsequent paper.

2,4-D had no apparent effect on thy-

TABLE 2. 2,4-D effect on 24-hour thyroidal I^{131} uptake after hypophysectomy and iodine depletion

	Rats per group	Controls	2,4-D-treated
Hypophysectomized	8	0.48 ±0.04%*	0.38 ±0.05%
Iodine Depleted	6	14.9 ±1.0%	14.8 ±1.2%

* Standard error of the mean.

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TABLE 3. Effect of 2,4-D on goitrogenesis due to 10 days of propylthiouracil feeding

	Controls	2,4-D-treated
Thyroid Weight	25.5 ±0.9* mg	25.8 ±0.6 mg
T:S Ratio	157 ±15	205 ±31
Number of Rats	6	6

* Standard error of the mean.

roidal I^{131} uptake in hypophysectomized rats, and in rats depleted of iodine by means of the Remington diet for two weeks there also was no effect of the drug on I^{131} uptake by the thyroid (Table 2).

Since the association of an increased thyroidal I^{131} uptake with a lowered serum PBI is found in nephrosis, we investigated this possibility, but kidney histology remained normal, there was no increased urinary protein loss, and the serum electrophoretic pattern was unaffected by 2,4-D administration. Iodide clearance, as judged by serum inorganic iodide-I^{131} levels 24 hours after tracer administration, also did not differ significantly from the control levels. When we attempted to measure other criteria of thyroid function, we found that many of these remained completely unchanged (Table 1). Thus, the thyroid cell height and hiotslogic appearance were not affected by 2,4-D, and neither was the rate of loss of radioiodine from the gland. When rats were kept on a goitrogenic diet for two weeks while being given 2,4-D daily, goiter development was unaffected and the terminal value of the thyroid:serum radioiodide concentration (T:S) ratio was not elevated significantly (Table 3). Pituitary TSH stores measured by McKenzie's assay (5) were unaffected by 2.4-D. Serum TSH levels could be measured only after concentrating pooled sera from 12 rats by Bates's procedure 6. We found levels of about 0.025 milliunits (mU)/cc of serum in both 2,4-D-treated and control rats (Table 4).

The iodinated amino acids of the thyroid glands of treated and control rats were analyzed 24 hours after radioiodine administration. Using the ion-exchange chromatographic system of Galton and Pitt-Rivers (7) on pancreatin digests, we obtained the results shown in Table 5. Total iodine analyses were performed on aliquots of the thyroxine fraction and the pooled iodotyrosine eluates, and the percentage of thyroxine among the total iodinated amino acids also was found to be unaffected by 2,4-D.

The results of the determination of the serum iodide level and PBI by the isotope equilibrium technique are shown in Table 6.

To confirm the magnitude of the serum iodide levels found, we calculated the difference between the serum total iodine and the serum protein-bound iodine levels in 11 control rats by chemical assay. This difference averaged 1.0 ±0.1 (standard error) μg/100 ml, in good agreement with the levels calculated from radioactivity data in the animals in the experiment of Table 6 that were on the same iodine intake.

TABLE 4. Pituitary and serum thyrotropin assays

Pituitary TSH Content;	
Controls (5)	93 ±15* mU per gland
2,4-D-Treated (5)	108 ±20 mU per gland
Serum Levels;	
Controls	0.028 mU/cc
2,4-D-Treated	0.024 mU/cc

* Standard error of the mean.

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TABLE 5. Analysis of thyroidal iodinated amino acids

	Controls	2,4-D-treated	Significance of difference (*P*)
Number of rats	12	12	
24-hr I^{131} uptake	6.9 ±0.8%	10.2 ±1.0%	<.02
Serum PBI	3.9 ±0.1 μg/100 ml	3.2 ±0.1 μg/100 ml	<.001
24-hr conversion ratio	30 ±2%	26 ±2%	.2
I^{131} Distribution:			
Thyroglobulin	4.9 ±0.4%	4.2 ±0.1%	
Iodotyrosines	62.6 ±2.4%	62.9 ±1.9%	
Iodothyronines	10.6 ±0.8%	11.7 ±0.9%	
I^{127} Distribution:			
Iodothyronines as fraction of total iodinated amino acids	19.4 ±0.7%	21.0 ±0.8%	>.1

Discussion

2,4-D has rather unusual effects on thyroid radioiodine uptake and serum PBI, the two most commonly used criteria of thyroid function. It is one of the very few compounds known that elevates I^{131} uptake. The only previously described situations in which similar results are obtained are nephrosis and iodine deficiency. In both of these there is evidence of increased thyrotropin secretion from the pituitary, whereas we could show no evidence of this after 2,4-D administration. Pituitary and serum thyrotropin assays support the evidence afforded by the unchanged thyroidal I^{131} release rate and by the histologic appearance of the glands and the epithelial cell height. In our hands, the index of precision of McKenzie's assay has averaged about .32, which allows for a large margin of uncertainty in the results. With the low levels of TSH found in serum, which we estimate at about 0.025 mU/cc, we could use only five mice per point, and this severely limited the accuracy of the assay. The TSH level we find in the serum of our rats is much lower than that which has been reported by D'Angelo, using the stasis tadpole assay (9).

In the present study, we found no differences between 2,4-D-treated and control rats with regard to the distribution of stable iodine and of radioiodine among thyroglobulin, iodinated tyrosines, and tri- and tetra-iodothyronine. We did not determine whether or not the iodide content differs, since this fraction was not separated from partial hydrolysis products and iodine bound to insoluble cellular debris.

The results of the 24-hour radioiodine uptake test, therefore, seem to conflict with a battery of other thyroid function tests. It appears that the enhancement of thyroidal I^{131} uptake must be caused by a change in the specific activity of serum iodide due to a lesser dilution of the injected carrier-free radioiodide. The isotope equilibrium experiments at both the 5 and the 10 μg per rat per day iodine intake level showed clear depression of the serum iodide level. The likelihood of radiation damage to the thyroid in these experiments is negligible, since the total radiation dose received by the glands was well below the 3,000 r threshold below which radiation damage has not been demonstrated (10). The iodide levels found were of the same order of magnitude as those calculated from the difference between chemically determined serum total iodine and the PBI, which is not in agreement with the work of Gerbaulet and collaborators

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... who believe iodide levels in human serum to be about one twentieth of the value calculated from the total iodine–PBI difference. However, it appears that Gerbaulet's assumption that saliva contains iodide in equilibrium with plasma iodide as the only iodine-containing compound may not be valid (12, 13).

It is of some interest that, in all the isotope equilibrium experiments we have carried out, the PBI level calculated from radioactivity data was consistently and significantly lower than the PBI determined on the same samples by chemical analysis using TCA precipitation. The average difference was 35%. A similar finding has been reported by Van Middlesworth (14). The significance of this discrepancy is under investigation. It does not appear to be due to a failure to achieve isotopic equilibrium during the 26-day experimental period. Using Pitt-Rivers and Rall's (10) method of calculating the period needed for achieving equality of specific activity of all iodinated materials in the animals from the turnover rate of thyroidal radioiodine after a single tracer dose, we found that 23 days should suffice to achieve 97% of equilibrium under our conditions, since the half-life observed was 4.0 to 4.6 days. However, Morel and Simon (15), using a much more laborious technique for determining the attainment of equilibrium, required 32 days to achieve 95% equilibration in rats that were considerably older than the ones used in Pitt-Rivers' and our studies. In experiments to be reported elsewhere, we have administered the long-lived isotope I^{129} for 35 days and still observed highly significant differences between the PBI calculated from radioactivity data and the chemically determined PBI, which were of the same magnitude as those recorded in Table 6. For these reasons, we believe that isotopic equilibrium was attained in our experiments and that other explanations must be sought for the discrepancies between the PBI values obtained by the alternate procedures. In any case, the determination of serum inorganic iodide levels, which was the object of these experiments, should certainly be valid since inorganic iodide equilibrates much more rapidly than the organically bound iodine.

Thus 2,4-D appears to be one of the few agents other than iodide itself that has been shown to alter the serum iodide pool specifically. Our results again emphasize the necessity of using a large number of thyroid function tests when exploring thyroid-pituitary interrelationships, since many peripheral factors, few of which have been explored adequately, can invalidate several of the commonly used tests.

TABLE 6. Isotope equilibrium determination of serum iodide and PBI

	Controls	2,4-D-treated	Significance of difference (*P*)
Experiment 1: 14 rats per group on daily dose of 5 μg iodide			
Serum Iodide	0.78 ±0.03* μg/100 ml	0.53 ±0.03 μg/100 ml	.005
PBI	1.92 ±0.10 μg/100 ml	0.87 ±0.08 μg/100 ml	<.001
Chemical PBI	3.3 ±0.1 μg/100 ml	1.7 ±0.1 μg/100 ml	<.001
Experiment 2: 14 rats per group on daily dose of 10 μg iodide			
Serum Iodide	1.48 ±0.08 μg/100 ml	0.92 ±0.04 μg/100 ml	<.001
PBI	1.92 ±0.07 μg/100 ml	1.35 ±0.09 μg/100 ml	<.001
Chemical PBI	3.2 ±0.1 μg/100 ml	1.9 ±0.1 μg/100 ml	<.001

* Standard error of the mean.

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Clinical Facts

Lille Medical

V. 7(10) 1049-51 (1962)

POLYNEURITIS AFTER USING A WEED KILLER: CONTAINING ACID 2-4-D.

By Philippe Foissac - Gegoux, Annie Lelievre, Bernard Basin and Pierre Warot.

By 1958, one of us (*) had already drawn attention on the risks involved with the more and the more frequent use of chemical products in agriculture: two observations were considered at the beginning of this work which concerned the dangers derived from handling organics derived from phosphorus, powerful insecticides; one of these observations concerned an agricultural worker who handled, many months before the installation of a polyradiculorephritis syndrome weed killers, sodium and

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potassium salts of 2- methyl -4- chlorophenoxyacetic acid; for several reasons, we had eliminated in this case the toxic origin of the neurological troubles..., but we ignored at that time that these products could have a toxic effect.

In truth, the cases of intoxication due to weed killers are rare and are especially known by toxicologists and doctors; but it is feared that they may multiply: for almost all the substances destined to stop the development of weeds are dangerous to man. Mineral compounds were being used such as chlorates and sulphuric acid: these were three times more inconvenient with an action often little selective, hence a global destruction of grasses and cereals, of a delicate use (risks of explosion and fire) and of a sure toxicity (burns-methemoglobinemy). The "vegetable hormones", the last commers, make up an undisputed progress. This name is given to chemical bodies whose action is comparable to that of phytohormones elaborated by the plant itself. In small doses, they in fact stimulate the growth, of young vegetable cells; but in the large doses that they are used they cause "a crazy exaltation of the manifestations of growth, a disorderly activity which drives the most deverse monstruosities, the intense consumption of reserves and finally the death of the grass".

These "hormones" are oxyacetic derivations of the benzine nucleus and of the naphtalene nucleus of the phenyl, naphtyl and indolacetic, propionic and butylic acids as well as some of their derivatives. One of the most widely used is 2-4-dichlorophenoxyacetic acid (Ac. 2-4-D.) whose action powerful and

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selective, is not, however, without inconvenience as the following observation proves.

Observation:

G... Gerard, 52 years old, farmer, was referred to the Neurology Clinic on July 30, 1962 by Doctor Chuffart for sensory and motor problems of the lower limbs. This man, without any previous pathological problem, who is not an alcoholic, dates the beginning of his troubles precisely on June 2, 1962. On that date he felt, suddenly, an acute pain throughout the entire right orbital and periorbital region becoming weaker the next day making room for (burning sensation) paresthesra and numbness in the same area. Also on June 3rd, he noticed a (decreased sensitivity) hypoesthesia in the right leg.

On June 16, two weeks later, the right leg recovered a normal sensitivity but the numbness was now felt on the left leg; besides, the lower left limb is now "weaker" and cannot move except with the aid of a cane.

The problems remained about the same untill July 30 when he entered the service. He now felt a persistant hypoesthesia in the right eye area, with hypoethesia of the cornea, the patient now felt some pain in the same area.

The subject is uncertain of his lower left limb where a slight reduction -global- of the segmentary force is noticed. There is no other trophic vaso-motor trouble.

The knee reflexes are rather sharp while the ankle reflexes are suppressed. The lower left limb suffers from a global

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hypoethesia only deep sensitivity was maintained.

The neurological examination of the upper limbs is strictly negative. Besides the general condition is excellent. There is no albuminuria. The blood formula reveals an eosinophilia (8% on Aug 2 and 9% Aug 30). Two electrophoretic blood tests, several weeks apart show a slight decrease in albumina (43 then 46 grams) and an increase of alpha -2- globulines (13 then 15 grams per liter).

The renal biological exam, the galactosuria provoked, the reactions of floculation do not show any abnormalities.

The electrocardiogram is normal. So is the backside of the eye and the E.E.G. The electrodiagnosis shows the following results: ——— proof of stimulation of Aug 2, 1962: integrity of the upper limbs; in the lower limbs there is no evident weakness but slight problems of excitability (galvanic hyperexcitability - galvanic and faradic hypoexcitability of the extensor of the left big toe —— high chronaxies of the extensors of the left big toe) witness however a slight neurogenic seizure (Doctor Spy); ——— electromyography of the two frontal tibia on August 4, 1962 (Doctor Ramez): trace of neurogenic seizure of the two muscles examined.

The patient leaves the clinic on September 8, 1962: during hospitalization he was submitted to a daily injection of 200mg of vitamin B1 and 250mg. of vitamin B6; besides 5,000 gammes of hydrocobalamine were administered every three days. The facial aches practically disappeared; the deficiency of sensitivity in

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the face and the lower left limb was notably improved; the motor deficiency disappeared and the patient was able to walk normally; the areflexia achilleine persists, there is no visible atrophy.

There are still electromyographic signs of neurogenic seizure of the right frontal tibia (the left side was not examined). On the contrary the electrodiagnostic of stimulation of August 28 are absolutely normal.

The aetiology of this polyneuritis of the lower limbs with irritative and deficient right trigeminus was suggested to us by the patient himself who was able to relate the beginning of his trouble while killing grass. Made June 1st and the last days of May 1962. This operation consisted of a spraying of two different solutions but both having a base of acid 2-4-D, containing 235 and 410 grams per liters. It was carried out with a tractor which tows the spraying device and which is in a cabin, open in the back; it was very windy when the operations took place and the wind beat the toxic substance in the cabin.

These exceptional circumstances, the long exposure to the toxic substance, we had to admit that it was a polyneuritis due to 2-4-D acid.

Besides, this observation is not alone in the litterature that includes a certain number of facts of intoxication by these substances, notably in the case of farmers: some hours after inhalation of the toxic substance there is generally vomiting,

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then subjective sensitive troubles of the type of paresthesis or even pain, then motor troubles, the paralysis of the four limbs or the lower limbs. This sensitive - motor polyneuritis —— inconstantly associated with an albuminuria and an intercolite board picture chart description —— is usually irreversible or very slowly and very partially reversible.

The toxicity of these substances is equally proven in the case of intoxication of workers at a vegetable hormone factory: The penetration of the toxic substance through inhalation, but also through the skin; the workers often complained of somnolence, of the lower limbs feeling heavy, gastralgia, loss of appetite, hypersialosis, as well as hypersensitivity of hearing: all of these troubles are, generally, transient. Finally, experimentally, 2-4-D acid administered intravenously or even orally can be fatal: this happens suddenly, either in a neurological stage in the form of a coma with hypertonic of the limbs, or ventricular fibrillation with relatively large doses, but varying according to the animal tested (about 300mg to one gram). To our knowledge, there was no acute intoxication.

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FAITS CLINIQUES

LILLE MEDICAL
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POLYNEVRITE APRES USAGE D'UN DESHERBANT : L'ACIDE 2-4-D.

par Philippe Fossac-Geaux, Annie Lejèvre, Bernard Basix et Pierre Warot.

DOW 1 714162

Déjà en 1958, l'un d'entre nous (*) avait attiré l'attention sur les risques que comportait l'utilisation de plus en plus fréquente en agriculture de produits chimiques : deux observations étaient à l'origine de ce travail dans lequel l'accent avait été mis sur les dangers de la manipulation des dérivés organiques du phosphore, insecticides puissants ; l'une de ces observations concernait un ouvrier agricole ayant manipulé, plusieurs mois avant l'installation d'un syndrome de polyradiculonévrite, des désherbants, sels sodique et potassique de l'acide 2-méthyl-4-chlorophénoxyacétique ; pour plusieurs raisons, nous avions éliminé dans ce cas l'origine toxique des troubles neurologiques..., mais nous ignorions à l'époque que ces produits pouvaient avoir une action toxique.

A vrai dire, les cas d'intoxication par des désherbants sont rares et sont surtout connus des toxicologues et des médecins du travail ; mais il est à craindre qu'ils ne se multiplient : car presque toutes les substances destinées à entraver le développement des mauvaises herbes sont dangereuses pour l'homme. On utilisait naguère des composés minéraux parmi lesquels les chlorates et l'acide sulfurique : ceux-ci avaient le triple inconvénient d'une action souvent peu sélective, d'où une destruction globale des herbes et des céréales, d'un emploi délicat (risques d'explosion et d'incendie) et d'une toxicité certaine (brûlures - méthémoglobinémie). Les « hormones végétales », dernières venues, constituent un progrès indiscutable. On donne ce nom à des corps chimiques dont l'action est comparable à celle des phytohormones élaborées par la plante elle-même. A doses faibles, elles stimulent en effet la croissance des cellules végétales jeunes ; mais aux doses fortes auxquelles on les emploie, elles provoquent « une exaltation folle des manifestations de croissance, une activité désordonnée qui entraîne l'apparition de monstruosités les plus diverses, la consommation intense des réserves et finalement la mort au stade herbacé ».

Ces « hormones » sont des dérivés oxyacétiques du noyau benzène et du noyau naphtalène des acides phényl, naphtyl et indolacétique, propionique et butylique, ainsi que de certains de leurs dérivés. L'un des plus employés est l'acide 2-4-dichlorophénoxyacétique (Ac. 2-4-D.) dont l'action, puissante et sélective, n'est cependant pas dénuée de tout inconvénient ainsi que le prouve l'observation suivante.

OBSERVATION :

G... Gérard, 52 ans, agriculteur, est adressé à la Clinique Neurologique le 30 juillet 1962, par le Docteur Chuffart pour des troubles sensitifs et moteurs des membres inférieurs. Cet homme, sans antécédents pathologiques notables, qui n'est pas éthylique, situe très précisément le début de ses troubles au 2 juin 1962. Il ressentit ce jour-là, brusquement, une douleur vive dans toute la région orbitaire et périorbitaire droite, s'atténuant dès le lendemain pour faire place à des paresthésies et à une anesthésie occupant le même territoire. Le 3 juin également, il note l'apparition d'une hypoesthésie de la jambe droite.

Le 16 juin, soit deux semaines après le début, la jambe droite a récupéré une sensibilité normale mais l'anesthésie, qui persiste à la face, a gagné la jambe gauche ; d'autre part, le membre inférieur gauche est « plus faible » et la marche n'est possible qu'avec l'aide d'une canne.

Les troubles restent sensiblement identiques jusqu'au 30 juillet, date de l'entrée dans le service. On constate alors la persistance d'une hypoesthésie dans le territoire de l'ophtalmique droit, avec hypoesthésie cornéenne ; le malade accuse encore dans ce même territoire quelques épisodes douloureux très passagers et d'intensité modérée.

Le sujet se dit incertain de son membre inférieur gauche au niveau duquel on constate une légère diminution — globale — de la force segmentaire. Il n'existe aucun trouble trophique, aucun trouble vaso-moteur.

Les réflexes rotuliens sont plutôt vifs tandis que les achilléens sont abolis. Le membre inférieur gauche est le siège d'une hypoesthésie globale, seule la sensibilité profonde étant conservée.

(*) P. Nayrac, M. Foxtan, P. Warot, J. Lescut et J. Delahousse : *Lille Médical*, 1958, 3, 3, 161-164.

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L'examen neurologique des membres supérieurs se révèle strictement négatif. D'autre part, l'état général est excellent. Il n'y a pas d'albuminurie. La formule sanguine révèle une éosinophilie (8 %, le 2 août et 2 % le 30 août). Deux examens électrophorétiques sanguins, à plusieurs semaines d'intervalle, montrent une baisse légère de l'albumine (43 puis 46 grammes) et une augmentation des alpha-2-globulines (13 puis 15 grammes par litre).

Le bilan biologique rénal, la galactosurie provoquée, les réactions de floculation ne montrent pas d'anomalies.

L'électrocardiogramme est normal. Il en est de même du fond d'œil et de l'E.E.G. L'électrodiagnostic donne les renseignements suivants :

— épreuve de stimulation du 2 août 1962 : intégrité des membres supérieurs ; aux membres inférieurs, il n'y a pas de réaction de ralentissement évidente mais les légers troubles de l'excitabilité (hyperexcitabilité galvanique - hypoexcitabilité galvanique et faradique de l'extenseur propre du gros orteil gauche - chronaxies élevées des extenseurs du gros orteil gauche) témoignent cependant d'une légère atteinte neurogène (Docteur Sey) ;

— électromyographie des deux jambiers antérieurs du 4 août 1962 (Docteur Ramez) : tracé d'atteinte neurogène des deux muscles explorés.

Le malade quitte la clinique le 8 septembre 1962 : durant l'hospitalisation il a été soumis à une injection quotidienne de 200 mg. de vitamine B 1 et 250 mg. de vitamine B 6 ; en outre, l'hydrocobalamine lui a été administrée à la dose de 5.000 gammas tous les trois jours. Les algies faciales ont pratiquement disparu ; les troubles sensitifs déficitaires de la face et du membre inférieur gauche se sont notablement atténués ; le déficit moteur a disparu et la marche est redevenue normale ; l'aréflexie achilléenne persiste ; aucune atrophie ne s'est dessinée.

Il persiste des signes électromyographiques d'atteinte neurogène du jambier antérieur droit (l'examen n'a pas été pratiqué du côté gauche). Par contre, les réponses à l'électrodiagnostic de stimulation, en date du 28 août, sont absolument normales.

L'étiologie de cette polynévrite des membres inférieurs avec atteinte irritative et déficitaire trigéménaire droite nous a été suggérée par le malade lui-même qui n'a pas manqué de faire le rapprochement entre le début de ses troubles et une opération de désherbage effectuée le 1er juin et les derniers jours de mai 1962. Celle-ci a consisté en une pulvérisation de deux solutions différentes mais toutes deux à base d'acide 2-4-D. et en contenant 235 et 410 grammes par litre. Elle s'est effectuée grâce à un tracteur qui remorque le pulvérisateur et qui est surmonté d'une cabine, ouverte à l'arrière ; or, les opérations se sont déroulées par vent violent et celui-ci rabattait dans la cabine le nuage de substance toxique.

Ces circonstances exceptionnelles, la longue durée d'exposition au toxique, nous ont fait admettre qu'il s'agissait vraisemblablement d'une polynévrite due à l'acide 2-4-D.

Au reste, cette observation n'est pas isolée dans la littérature qui comporte un certain nombre de faits d'intoxication par ces substances, notamment chez des agriculteurs : quelques heures après l'inhalation du toxique surviennent habituellement des vomissements, puis des troubles sensitifs subjectifs à type de paresthésies ou même de douleurs, puis des troubles moteurs, l'atteinte paralytique pouvant intéresser les quatre membres ou les membres inférieurs. Cette polynévrite sensitivo-motrice — inconstamment associée à une albuminurie et à un tableau d'entérocolite — se révèle habituellement irréversible ou très lentement et très partiellement réversible.

La toxicité de ces substances est également prouvée par les cas d'intoxication chez les ouvriers travaillant à la fabrication des hormones végétales : la pénétration du toxique se ferait par inhalation, mais aussi par voie cutanée ; les ouvriers se plaignent souvent de somnolence, de sensations de lourdeur des membres inférieurs, de gastralgies, d'anorexie et d'hypersialorrhée, ainsi que d'une hypersensibilité de l'ouïe : tous ces troubles sont, dans la règle, fugaces. Enfin, expérimentalement, l'acide 2-4-D., administré par voie intraveineuse ou même par voie orale, peut entraîner la mort : celle-ci survient, soit dans un tableau neurologique sous forme de coma avec hypertonie des extrémités, soit par fibrillation ventriculaire, et ce pour des doses relativement élevées, mais évidemment variables suivant l'animal en expérience (de l'ordre de 300 mg. à un gramme en une prise). A notre connaissance, il n'a pas été effectué d'intoxication subaiguë.

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* *

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DOW 1 714164

MENINGITE A LISTERIA MONOCYTOGENES DE L'ADULTE. UN CAS DE GUERISON

Pierre Warot, Jacques Desax, René Lesage et Michel Dorchies.

Longtemps considérée comme l'apanage presque exclusif de l'animal, l'infection listérienne semble se manifester chez l'homme avec une fréquence accrue : d'après Benazet et Sohier, 70 cas seulement de listériose humaine avaient été rapportés avant 1950, tandis que 300 nouveaux cas l'étaient de 1950 à 1957.

Sans doute le diagnostic bactériologique est-il fait plus souvent qu'autrefois : « on passe souvent à côté du diagnostic bactériologique parce que l'on écarte trop vite, sous l'appellation de facilité de « contaminants diphtérimorphes » des bacilles Gram positif isolés d'hémocultures, de liquide céphalo-rachidien ou de tout autre produit et qui sont d'authentiques listéria » (Larcyssonnie). Mais il est très vraisemblable aussi que la diffusion, reconnue, de l'infection dans le règne animal entraîne la multiplication des transmissions accidentelles à l'homme.

Il est rare que le clinicien fasse le diagnostic de listériose et en demande confirmation au biologiste : rien d'étonnant à cela si l'on considère qu'il s'agit d'une infection de connaissance récente et dont la fréquence est d'ailleurs toute relative : la littérature française ne comportait en effet, en 1959, que 23 observations. D'autre part, les listérioses se présentent sous des aspects cliniques extrêmement divers : l'importante statistique de Seliger, portant sur les 344 cas publiés dans le monde en 1956, relève 160 états septicopyohémiques du nouveau-né, 100 méningites purulentes avec ou sans encéphalite, 41 formes sanguines à type de mononucléose infectieuse, 13 conjonctivites granulomateuses, les autres cas concernant des localisations viscérales diverses : endocardites, myocardites, métrites, etc...

Tout au plus peut-on parfois soupçonner l'origine listérienne d'un état septicopyohémique du nouveau-né, ou d'une méningite purulente de l'enfant ou de l'adulte, parce qu'il s'agit là des manifestations les plus fréquentes de l'infection. Mais, le plus souvent, le mérite du diagnostic revient au seul bactériologiste... et ce n'est qu'a posteriori, lorsque le germe est identifié, que le clinicien mettra l'accent sur quelques nuances séméiologiques qui auraient dû faire évoquer la listériose, mais dont la signification lui avait échappé.

C'est ainsi que les choses se sont passées dans l'observation que nous rapportons d'une méningite purulente de l'adulte, inquiétante d'emblée de par l'intensité des troubles de la conscience et du déréglement neuro-végétatif : ce fut une surprise d'apprendre que le germe responsable en était listéria monocytogène.

OBSERVATION.

M. Léon, 60 ans, mécanicien, sans antécédent pathologique notable, présente, le 17-3-1962, très brutalement, vers 12 heures, un état infectieux d'allure grippale, avec fièvre à 39°, courbures, malaise général, qui l'oblige à s'aliter. Ces troubles s'intensifient les jours suivants tandis qu'apparaissent céphalées de plus en plus intenses et vomissements.

Le 22 mars est constatée une raideur méningée et le malade est adressé à la Clinique Neurologique. Il est admis au début de l'après-midi. L'un d'entre nous constate alors un syndrome méningé franc, un état d'obnubilation intellectuelle avec légère agitation psychomotrice : la température est à 39°5 ; le malade a un hoquet permanent ; l'examen neurologique ne révèle aucun signe particulier. Le liquide rachidien se révèle

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Pathophysiologia

NEW EVIDENCE CONCERNING THE NERVOUS SITE OF ACTION OF A CHEMICAL HERBICIDE CAUSING PROFESSIONAL INTOXICATION

By

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INSTITUTE OF PATHOPHYSIOLOGY, MEDICAL UNIVERSITY, BUDAPEST

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The effect of 2,4-dichlorophenoxy-acetic acid on the electroencephalogram has been studied in thyroidectomized and thyroxine-substituted cats. A decrease of frequency and an increase of amplitude have been found to result as early as 24 hours after the onset of treatment. After 5 days, 2 to 3 Hz, 180 to 190 μV, big, slow, toxic waves predominated. Desynchronisation in response to stimulation of the reticular formation was significantly shortened, or even extinguished.

The primary site of action of DCPA is thought to be in the cerebral cortex. The disturbances of thyroid and cardiac activity were ascribed to lesions in various subcortical centres.

The noxious effects of 2,4-dichlorophenoxy-acetic acid have been under detailed study at our Institute [5, 6, 13, 14], in view of their significance in labour hygiene. Dichlorophenoxy-acetic acid (in the following DCPA) is a weed killer used in increasing amounts mainly in corn fields.

It has been shown by us that chronic treatment with DCPA caused very slow, 2 to 3 Hz, high amplitude waves to appear on the electroencephalogram and poisoning with the substance induced disturbances [5, 6] in thyroid function [13].

As changes in thyroid function are known to produce electroencephalographic changes [8], the question arose whether DCPA damaged the nervous system directly, or the changes observed in the EEG were due to an action on thyroid function.

This problem has been investigated in the present experiments.

Methods

A total of 15 cats, 11 experimental and 4 controls, weighing 2.5 to 3.5 kg, were used. The electrical activity of the central nervous system was studied in each animal by means of 2 frontal and 2 occipital cortical built-in electrodes, as well as bipolar subcortical electrodes placed in the mesencephalic reticular formation. To place the cortical electrodes, openings 2 mm in diameter had been cut in the calvarium and the silver electrodes were placed on the dura. To insert the subcortical electrodes, an opening 4 mm in diameter was made, the dura was incised, the silver electrodes enclosed in a glass tube were inserted into the reticular formation by means of a stereotactic apparatus. The coordinates were determined according to Szentágothai [9] After inserting the electrodes a fibrin sponge was placed in the drill hole. The location of the deep electrodes was controlled histologically after the experiment.

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The electrodes were cemented in place with acrylate.

After EEG tracings had been recorded from the otherwise intact cats, they were subjected to thyroidectomy under aseptic conditions. Subsequently, the controls were not treated in any way. The experimental animals were given 100 μg/kg thyroxine *(Hoffmann—La Roche)* daily.

We then studied for 10 days the experimental group to determine whether changes in the EEG would occur. No appreciable changes having been noted, from the 11th day on the experimental animals were given 100 mg/kg of DCPA daily. The material was obtained after recrystallising three times the commercial preparation.

Using a 4-channel EEG apparatus, the spontaneous electrical activity of the cortex and reticular formation was recorded daily.

We also studied the changes in the duration of cortical desynchronization in response to the electrical stimulation of the reticular formation.

The apparatus used for stimulation emitted rectangular impulses, with the parameters 0.2 V and 1.5 V, frequency 300 Hz, duration 2 msec. Total duration of stimulation was 5 sec.

Considering that the stimulations were applied once a day, no adaptation could influence the responses according to data in the literature [2, 4].

The cardiac rate was determined daily.

Results

The results obtained may be outlined as follows. In the control group the pre-thyroidectomy average frequency of 6 to 7 Hz decreased 24 hours after thyroidectomy and remained at a low level throughout.

In the experimental group no appreciable change in frequency was noted after thyroidectomy. On the 10th day after thyroidectomy the frequency ranged from 5.5 to 6.5 Hz, as compared with the pre-thyroidectomy values of 6 to 7 Hz.

On the other hand, frequency decreased 24 hours following the administration of DCPA and after 5 days it was as low as 2 or 3 Hz (Fig. 1).

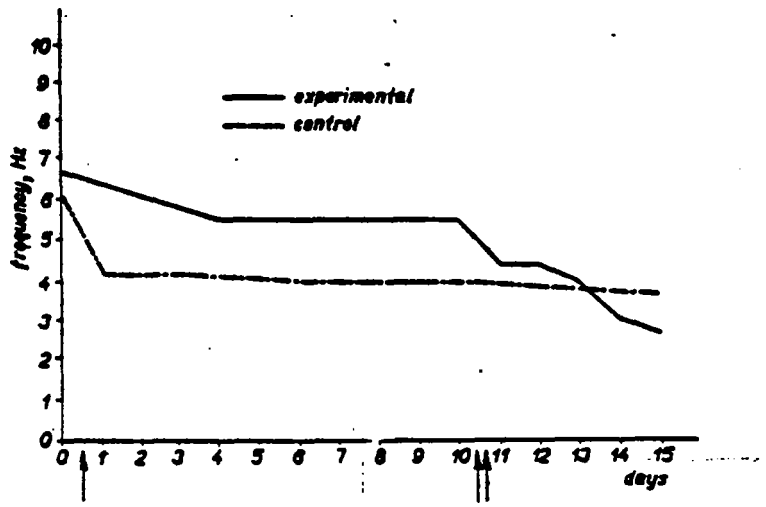


Fig. 1. Changes in the frequency of spontaneous cerebral electrical activity in the control and the experimental group
Arrow: thyroidectomy. Double arrow: onset of DCPA treatment

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Similar changes were in the amplitude of the waves (Fig. 2). In the control group the 60 to 70 μV amplitudes slightly increased after thyroidectomy, then remained at the 90 to 100 μV level throughout.

In the experimental animals the initial values were the same and 10 days after thyroidectomy they were still at the 60 μV level.

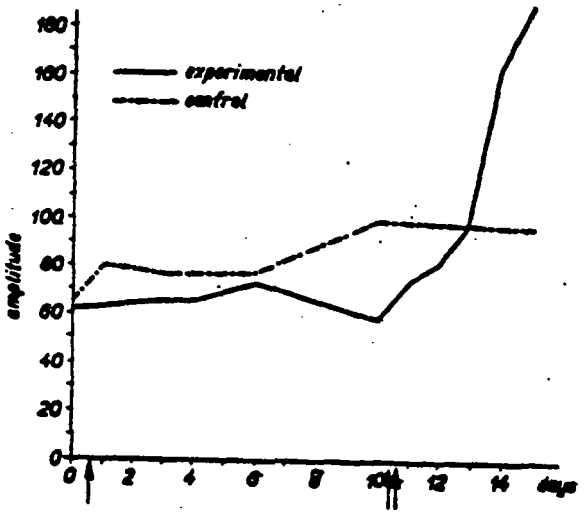


Fig. 2. Changes in the amplitude of spontaneous cerebral electrical activity. Signs as in Fig. 1
Ordinate: amplitude in μV. Abscissa: days

Twenty-four hours after the onset of DCPA treatment the amplitude increased parallely with the decrease of frequency. On the third day the average amplitude was 100 μV, and by the fifth day it reached values around 180 to 190 μV.

In the EEG tracings of the controls (Fig. 3), 3 days after thyroidectomy there was a significant slowing down (Fig. 3/II), as related to the initial value (Fig. 3/I). The rhythm was still slower 11 days (Fig. 3/III) and 15 days (Fig. 3/IV) following thyroidectomy.

In the experimental animals (Fig. 4) there was practically no EEG change after thyroidectomy (Fig. 4/II), as compared with the pre-thyroidectomy tracings (Fig. 4/I). Twenty-four hours following the onset of DCPA administration frequency decreased and the amplitude increased at sites (Fig. 4/III). After 5 days the tracings displayed exclusively big, slow, toxic waves (Fig. 4/IV).

The duration of desynchronisation in response to stimulation of the reticular formation showed characteristic changes (Fig. 5, Fig. 6); the value registered before thyroidectomy was taken to be 100 per cent, later deviations were calculated on that basis.

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In the control group the duration of desynchronization rapidly decreased after thyroidectomy. From the 6th day on no desynchronization resulted in response to stimulation with 0.2 V. From the first day on the duration of desynchronisation elicited by stimulation with 1.5 V was markedly reduced.

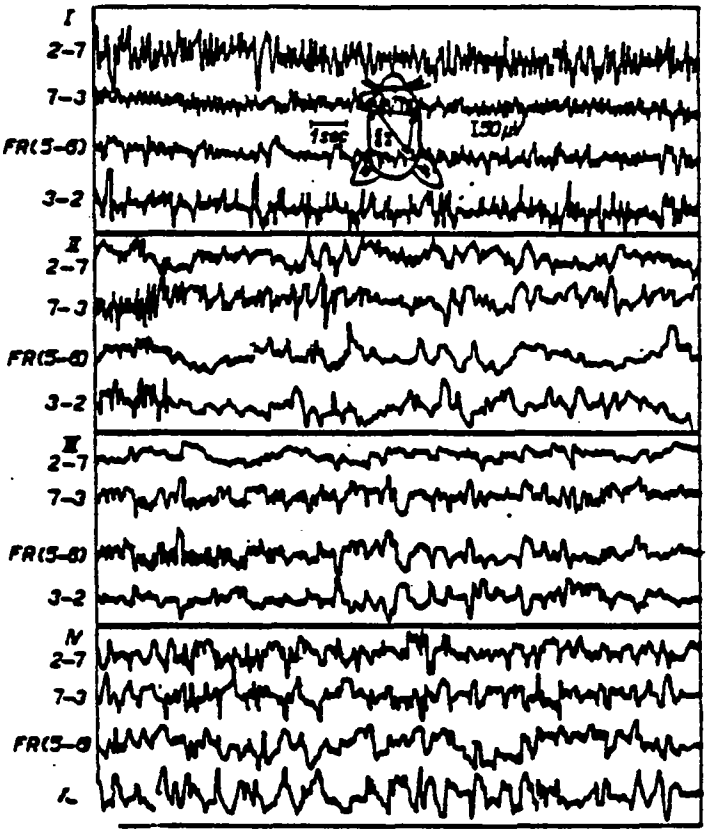


Fig. 3. EEG of control group. Diagram: site and connexion between electrodes. Numerals: No. of single electrodes. FR: reticular formation lead

I. Prior to thyroidectomy, normal activity. II. 3 days after thyroidectomy. Slowing, increase of amplitude, dysrhythmic curve. III. 11 days after thyroidectomy, and IV. 15 days after thyroidectomy: slowing and dysrhythmia slightly increased

In the experimental group desynchronisation time was reduced by 16 per cent on stimulation at 0.2 V 10 days after thyroidectomy. The duration of desynchronisation decreased rapidly on DCPA administration, after 24 hours by 50 per cent already, and on the fifth day no desynchronisation at all resulted on stimulation at 0.2 V.

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Ten days after thyroidectomy the desynchronization elicited by 1.5 V stimulation was reduced by 13 per cent. The duration of desynchronization rapidly decreased in response to DCPA, to 45 per cent by the 5th day.

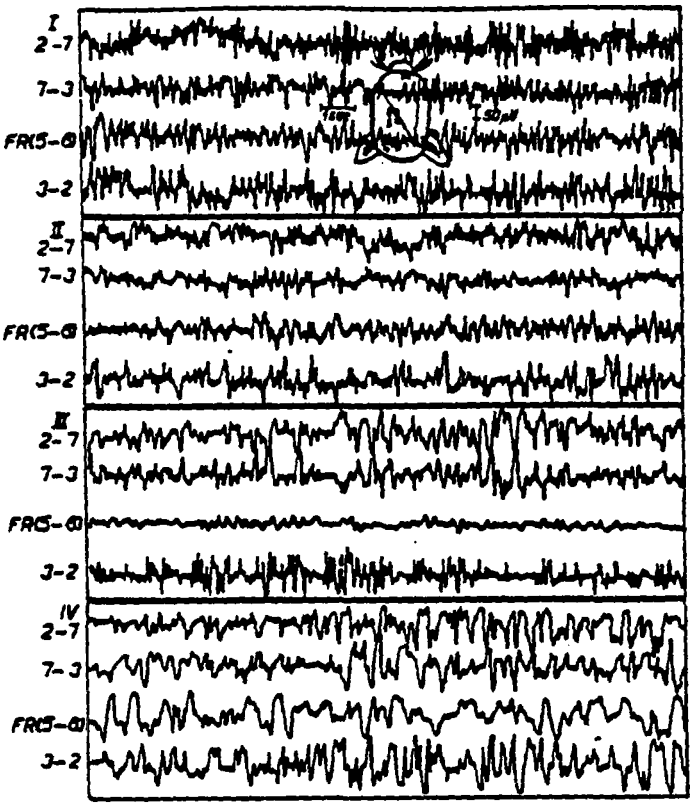


Fig. 4. EEG of experimental group. Signs as in Fig. 3
I. Normal activity before thyroidectomy. II. Normal activity 10 days after thyroidectomy III. 24 hours after onset of DCPA treatment. Slowing and at sites increase of amplitude. IV. 5 days after onset of DCPA treatment. Exclusively big, slow, toxic delta waves are visible

Changes have been noted in the cardiac rate, too (Fig. 7). In the control group thyroidectomy was followed by a gradual decrease, in the experimental group the heart rate decreased by 5 per cent in 10 days after thyroidectomy. Following the onset of DCPA treatment the decrease was 25 per cent after the first day and on the 5th day it was 36 per cent.

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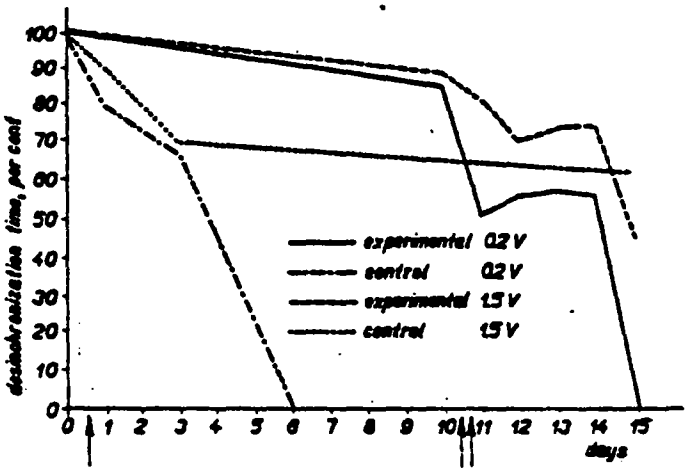


Fig. 5. Changes in the duration of cortical desynchronisation after electrical stimulation of the reticular formation. Graphical representation. Duration before thyroidectomy: 100 per cent. Difference from this given in per cent. Arrow: thyroidectomy. Double arrow: onset of DCPA treatment

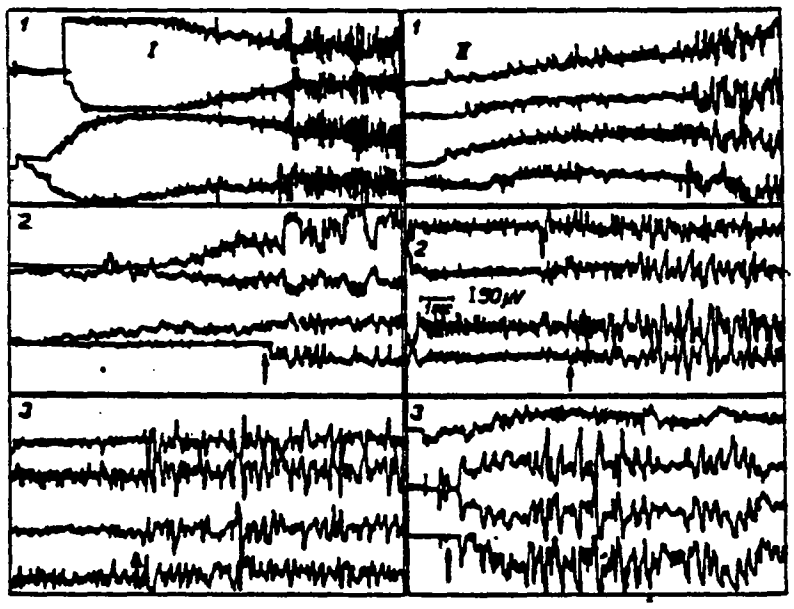


Fig. 6. Changes in the duration of cortical desynchronisation following stimulation of the reticular formation with 1.5 V. Arrow: the fast, low-amplitude desynchronisation ceases and the basal activity reappears.

I.: Experimental group. II: Control group. I/1. —/.. before thyroidectomy. I/2: Ten days after thyroidectomy. Desynchronisation time hardly shortened. I/3: 5 days after onset of DCPA treatment. Duration of desynchronisation strongly reduced. II/2: 5 days, II/3 15 days, after thyroidectomy. Duration of desynchronisation strongly reduced

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Discussion

According to the above data, the EEG changes induced by DCPA were identical in thyroidectomized animals maintained with thyroid substitution and in animals with intact thyroid [5, 6].

The appearance of 2 to 3 Hz, over 100 μV, big, slow waves (which are definitely in the pathological zone) is indicative of a severe toxic state, and together with the shortening of the duration of desynchronization it points to a marked reduction of central nervous excitability.

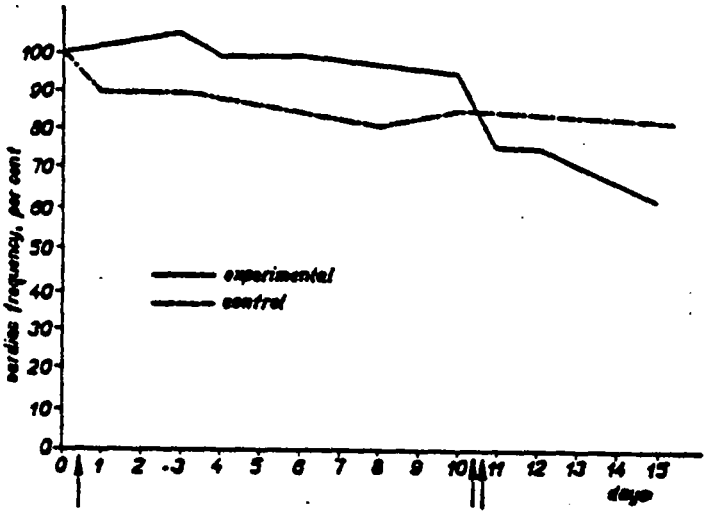


Fig. 7. Changes in heart frequency. Pre-thyroidectomy frequency: 100 per cent. Difference from this given in per cent
Arrow: thyroidectomy. Double arrow: onset of DCPA treatment

Comparison with controls has made it clear that the slowing down and the diminution of excitability were not due to thyroidectomy.

In the control group thyroidectomy was immediately followed by a slowing of frequency, an increase of amplitude and a diminution of excitability. These fit well into the pattern of the EEG in hypothyroidism, as reported in the literature [8].

During the 10 days of thyroid substitution these changes did not occur in the experimental animals, except for a slight, not significant slowing down.

The changes clearly observable as soon as 24 hours after starting DCPA treatment prove that they had been produced exclusively by that treatment.

Thus, it may be assumed that the EEG changes noted in the animals with intact thyroid were not due to alterations in thyroid function.

The changes in the reticular formation, as determined by EEG, were just as severe as those in the cortex. The cortical desynchronisation in response

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to the stimulation of the reticular formation was shortened in duration, or disappeared altogether.

According to data in the literature [1, 7, 11, 12], when stimuli capable of eliciting cortical desynchronisation lose this ability, it is usually due to a functional block in the reticular formation. Thus, our findings may be explained by a damage to the structures of the reticular formation.

Accordingly DCPA seems to produce lesions at the cortical and subcortical level in the central nervous system. It may be assumed that the nervous site of action is the primary one, as the nervous symptoms appear sooner than the symptoms indicative of a disturbance of thyroid function. It may be surmised that subcortical centres other than the reticular formation are also damaged, and their lesion may explain the disturbance of thyroid function.

Lesion to subcortical centres may also be responsible for the reduction in heart rate.

Under the experimental conditions employed it cannot be ruled out completely that in DCPA poisoning the organism's thyroxine requirement increases and thus what we have on hand is a relative hypothyroidism.

It can be brought up against this view among others that the 15 days of total thyroidectomy in the control group produced much slighter changes than did the few days of DCPA treatment in the experimental group.

It cannot be ruled out with certainty, either, that the EEG pattern was influenced by changes in the function of other organs.

Nevertheless, on the basis of the results obtained it is believed that DCPA acts primarily on the central nervous system, paralysing its function. The functional disorders in different organs are merely secondary to the central nervous effect.

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Orvostudományi Egyetem Kórélettani Intézete, Budapest, IX., Hőgyes Endre u. 9.

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AUTHOR: Desi, I; J. Sos, and I. Nikolits

SUBJECT: Chemical herbicide

TITLE: New evidence concerning the nervous site of action of a chemical herbicide causing professional intoxication.

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I. TOXICOLOGICAL PROPERTIES OF TORDON*

Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan
January 15, 1963

CONTAINS TRADE SECRET OR OTHERWISE CONFIDENTIAL INFORMATION
THE DOW CHEMICAL COMPANY

DOW 526671

SUMMARY AND CONCLUSIONS

Acute oral toxicity studies were conducted on several species of animals. In each case the material was fed by single dose intubation as a suspension or slurry in corn oil. A summary of the acute oral toxicity data appears below:

Animal	100% Survival g./kg.	100% Fatal g./kg.	LD_{50} g./kg.
Chick (M)	3.98	7.95	approx. 6
Mouse (F)	1.00	7.95	between 2 and 4
Rabbit (Mixed sex)	1.0	3.98	approx. 2
Cavy (F)	2	3.98	approx. 3
Rat (F)	---	----	8.2 - range (6.3-10.7) g./kg. body weight**

** Calculated by the Weil Modification of the Method of Thompson.

Undiluted Tordon when applied directly to the conjunctival sac of the laboratory rabbit produced slight to moderate redness of the conjunctiva which subsided between 48 hours and 1 week. The material was essentially nonirritating to the cornea.

Tordon was applied to the shaved, abraded and intact rabbit belly on a repeated, prolonged, confined basis, (continuous contact under a bandage for 11 days to intact skin and 3 days to abraded skin). Under this conditions of rather drastic exposure, the material produced essentially no skin irritation. Tordon was studied for skin absorption properties by applying it with Dowanol DPM in graduated doses to rabbit skin under an impervious cuff. Judging by weight gain and general appearance of the animals, Tordon is not absorbed in acutely toxic amounts.

* Trade Name for 4-Amino-3,5,6-Trichloro Picolinic Acid

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SUMMARY OF TOXICOLOGICAL DATA

Acute Oral Toxicity

Animal	Preparation Fed	Dose (g./kg.)	No. Died No. Fed	Response-Remarks
Chick	Capsules	3.98	0/2	
Chick	Capsules	7.95	3/3	Animals died overnite.
Mouse	20% suspension in corn oil	1.0	0/2	
Mouse	20% suspension in corn oil	2.0	1/2	Animal died 2 days after feeding.
Mouse	20% suspension in corn oil	3.98	1/2	Animal died within 2 hours after feeding.
Mouse	20% suspension in corn oil	7.95	2/2	Same as above.
Rabbit	39.8% slurry in corn oil	1.0	0/2	Animals appeared normal during and after feeding.
Rabbit	39.8% slurry in corn oil	2.0	1/2	Animal died 3 days after feeding.
Rabbit	39.8% slurry in corn oil	3.98	2/2	Same as above.
Cavy	20% suspension in corn oil	2.0	0/2	Animals appeared normal during and after feeding.
Cavy	20% suspension in corn oil	3.98	2/2	One animal died overnite the other three days later*
Rat	39.8% slurry in corn oil	6.3	2/5	One animal died 4 days after feeding the other 8 days.
Rat	39.8% slurry in corn oil	7.95	1/5	Animal died 3 days after feeding.
Rat	39.8% slurry in corn oil	10.0	4/5	One animal died 3 days after feeding the other 10 days.
Rat	39.8% slurry in corn oil	12.6	5/5	Animals okay in one hour but had diarrhea the following day.

* Animal was fed 1/2 dose in the morning and 1/2 dose in the afternoon.

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Eye Contact - Rabbit

Material	Treatment	No. of Animals	Response-Remarks
Undiluted	Unwashed	3	Very slightly painful. Slight to moderate conjunctival redness subsided in 48 hours to one week.
Undiluted	Washed with water	3	Washing enhanced recovery in 2 of the 3 animals tested.

Skin Contact - Rabbit

Material	Condition of Skin	No. of Appl.	Site	No. of Animals	Response-Remarks
Undiluted	Intact	9	Belly	3	No irritation observed.
Undiluted	Abraded	3	Belly	3	Essentially no irritation observed. Healing appeared to be normal in all cases.

Skin Contact Absorption - Rabbit (Cuff Technique)

Material	Duration of Exposure	Dose (g./kg.)	No. Died / No. Exposed	Response-Remarks
Undiluted	24 hours	1.0	0/2	Material colored skin reddish-brown, otherwise animals appeared normal during and after exposure.
Undiluted	24 hours	2.0	0/2	Same as above.
Undiluted	24 hours	3.98	0/3	Exposure was made to intact skin. Response was essentially the same as above.
Undiluted	24 hours	3.98	0/3	Abrasions were made the full length of the body 1-2 centimenters apart. Response was essentially the same as above.

NOTE: Material was weighed out for each rabbit and slurried with a small amount (8-9 cc.)of Dowanol DPM (Dipropylene glycol methyl ether).

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2,4-D K-337-171

Nervous System Effects of a Chemical Herbicide

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PROF. J. SOS, M.D.
J. OLASZ, M.D.
F. SULE
AND
V. MARKUS
BUDAPEST, HUNGARY

Thousands of new compounds are being encountered in man's environment all over the world. The biological effects of changes in the chemical environment are beginning to appear. Newly synthesized substances are causing different pathological processes. It is necessary, therefore, to become acquainted with the damaging effects of new substances on the human organism in order to devise protection against them.

The chemical herbicides are one of a group of the increasingly used compounds.[8] Among them, 2,4-dichlorophenoxy acetic acid (hereafter designated 2,4-D) plays an important role. This compound was synthesized in 1944 by Zimmerman, and its utilization as a chemical herbicide was proposed by Mitchell and Marth.[9] In Hungary it is called Dikonirt and has been made use of to an increasing degree since 1952.[1] Its molecule is similar to that of tryosine. It exerts an effect similar to plant hormone. It causes disproportionate growth and quick death of certain plants. It has a selective effect on different plant species. Among the monocotyledonous domesticated plants, 2,4-D kills the dicotyledonous weeds.[1,12]

In 1946 Hildebrand[8] was of the opinion that peroral 2,4-D had no pathogenic effect on either experimental animals or man. However, not much later Bucher[4] and Hill[7] found myotonia, motor disorders, paralyses in the extremities, and gastrointestinal symptoms such as vomiting and diarrhea in experimental animals treated with this compound. Others observed leukopenia,[5] ataxia and coma[10] after experimental intraperitoneal administration of large doses of 2,4-D.

Most recently Goldstein[6] reported on 3 patients in whom grave sensory and motor symptoms, paresthesias, paralysis, and severe pain appeared due to the percutaneous effect of 2,4-D. Recovery was only partial even after years.

In Hungary, Bordás[1-3] dealt with the problem. He found weight loss and symptoms like those mentioned above in his animals but could not demonstrate any objective changes in workers spraying 2,4-D. Sós et al.[11,12] have been examining the effect of 2,4-D on thyroid function using radioiodine and increased activity has been detected.

However, none of these studies has dealt with the problem of whether or not 2,4-D has a damaging effect on the function of the central nervous system. The present experiments undertook to study this question.

Methods

Thirty-two white male rats weighing about 240-250 gm., 5 cats, and 2 dogs were used in these experiments. The electrical activity of the central nervous system was examined by the use of electroencephalograms in acute and chronic

Submitted for publication June 20, 1961.
From the Institute of Pathophysiology (Director: Prof. J. Sós) of the University Medical School, Budapest, Hungary.

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exposures. Two frontal and 2 occipital electrodes were placed on the cerebral cortex, and a bipolar subcortical electrode was placed on the reticular formation. In the acute experiments the animals were anesthetized with 40 mg. of pentobarbital (Nembutal) per kilogram of body weight, and the electrodes were placed on the cortex and into the subcortex after removal of calvaria. In the chronic experiments the electrodes were fixed to the top of the skull with dental cement and acrilate, and the electroencephalograms were taken on unanesthetized animals moving without limitation. The spontaneous electrical activity of the animal's brain as well as response measured in cerebral electric activity to sound and electrical stimuli were recorded.

A whistle lasting for 5 sec. was used as the sound stimulus. For the electrical stimulation a square-wave impulse generator was used. The parameters of the square waves were: voltage 1 volt, frequency 100 per second, duration of impulse 8 msec. The entire duration of the stimulation was 5 sec.

To examine higher nervous activity, conditioned-anxiety-reflex experiments were performed with the method elaborated in our Institute.[18] Electric shock was applied as the unconditioned and light as the conditioned stimulus. 2,4-D furnished by Bordás was administered in its pure form. In the acute experiments a dose of 200 mg. per kilogram of body weight was administered intraperitoneally on a single occasion. The same amount was given daily in the chronic experiments until the death of the animals. In the experiments in which cortical application was used, some crystalline granules of the drug were placed upon the exposed cerebral cortex. Tyrosine was used for the controls.

The brain and spinal marrow of the animals were examined histologically at the end of the experiments.

Experimental Results

It is well known that if a normal animal under complete rest undergoes stimulation, the frequency of cerebral electric activity will immediately rise and the amplitude decrease with resumption of the original activity some time after cessation of the stimulation. This phenomenon is the so-called "desynchronization." Any chemically active agent which induces the inhibition of cere-

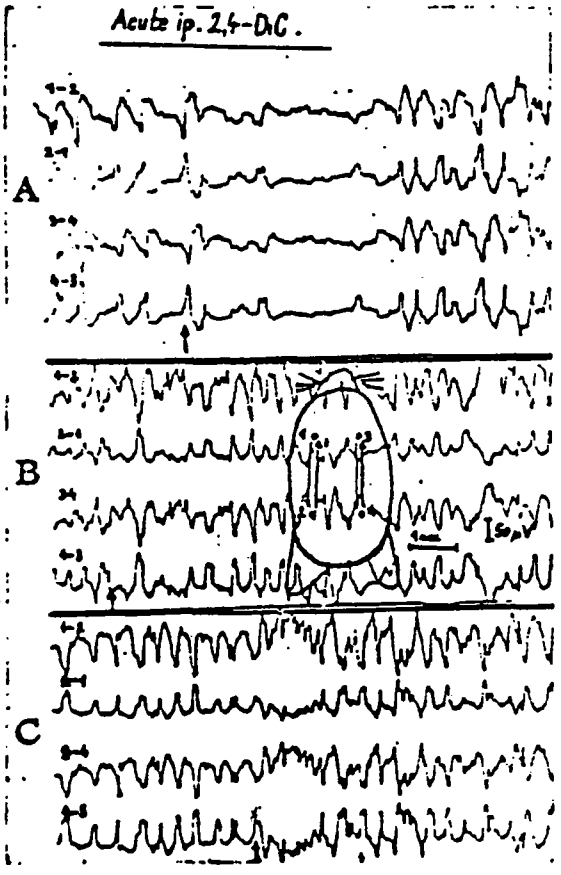


Fig. 1.—EEG curve of sleeping rat. Sound stimulus was applied at arrow. *A*, prior to treatment. *B*, thirty min. after injecting 200 mg. per kilogram of body weight of 2,4-D; no desynchronization. *C*, after 60 minutes; desynchronization appears due to sound stimulus. The drawing of the head shows the location and connection of each electrode. The numbers shown are the number of electrodes.

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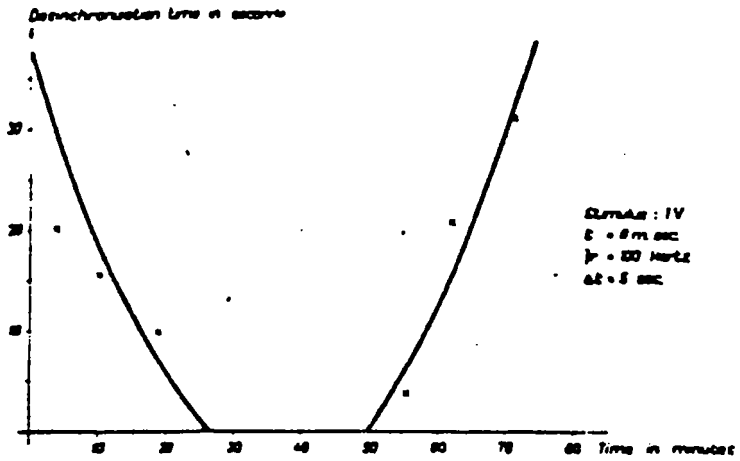


Fig. 2.—Effect of electric stimulation on the duration of desynchronization. The abscissa shows time elapsed since the injection of 2,4-D, expressed in minutes. The ordinate shows the duration of desynchronization in seconds.

bral function and decrease in the function of nerve cells slows the spontaneous and desynchronization frequency, shortens the duration of desynchronization, and possibly abolishes it completely. Every chemical or sensory influence causing excitement of the neurons accelerates the frequency and decreases the amplitude. Desynchronization is also a state of excitement after one or another stimulus.

In the first series of experiments the acute effect of the injection of 2,4-D into 8 rats was observed.

In untreated animals desynchronization manifested by response to the sound stimulus could no longer be elicited 10-15 min. after injection of the drug. This inhibition lasted 60 min. and was followed by a normal desynchronization in response to the sound stimulus (Fig. 1).

A similar change could be observed due to electrical stimulation of the reticular formation (Fig. 2). In untreated animals a desynchronization lasting 40 sec. occurred in response to the stimulus. After the injection of 2,4-D the duration of desynchroni-

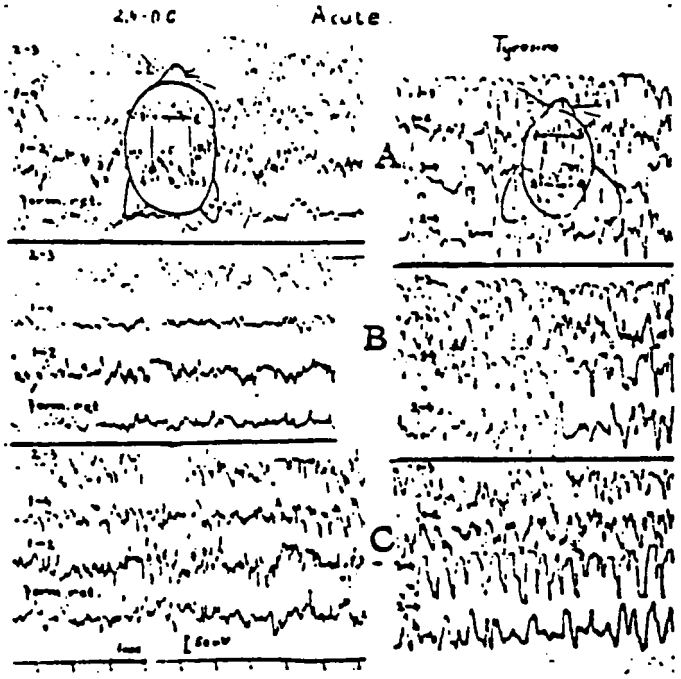


Fig. 3.—EEG effect of 2,4-D (left) and tyrosine (right) placed on the cortex: 2,4-D between the electrodes 1-4; tyrosine between the electrodes 1-2. *A*, prior to placing on the substances. *B*, 25 minutes afterwards: with 2,4-D a marked decrease in amplitude between the electrodes 1-4; with tyrosine no change whatever. *C*, after 40 minutes; normal electric activity resumed; *form. ret.* indicates reticular formation.

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zation after electrical stimulus became shorter, and, on the average, at the twenty-fifth minute after injection, desynchronization was abolished. After the lapse of 50 min. the response could be elicited again, and at the seventieth minute it reached the duration characteristic of the period prior to injection of the drug.

In the next series of acute experiments performed on 8 rats and 1 cat, some granules of crystalline 2,4-D and, in the controls, crystalline granules of tyrosine were placed directly on the cortex.

In response to 2,4-D the EEG curves 10-15 min. after application of the drug showed a marked decrease in amplitude and an increase in frequency. The change demonstrated itself only locally between the 2 electrodes between which the 2,4-D had been placed. At other sites of the cerebral cortex and in the reticular formation there was no change in electrical activity. The amplitude resumed its original size after 35 min. on the average. Tyrosine, applied in the controls, induced no change (Fig. 3).

In the next experimental series chronic experiments were performed on 8 rats, 4 cats, and 2 dogs.

During the chronic experiments the frequency of spontaneous electric activity showed a steady slowing. Instead of the waves having an average frequency of 7 per second as prior to treatment, there were large slow waves of 2-per-second frequency on the fifth day after injection. The quick waves appearing with desynchronization slowed down from 12.5 to 4.5 per second by the fourth day. Also the duration of desynchronization was moderated day after day. Prior to treatment it averaged 32 sec. After the lapse of 24 hr. the duration was only 21.2 sec., a 34.2% decrease. On the second day of treatment the duration of desynchronization was 14 sec., a 54.5% decrease. On the third day the duration of desynchronization was 6 sec., an 81.2% decrease. On the fourth day this duration was only 4.1 sec., a 91.4% decrease. On the fifth day there was no desynchronization at all in response to the sound stimulus (Figs. 4 and 5).

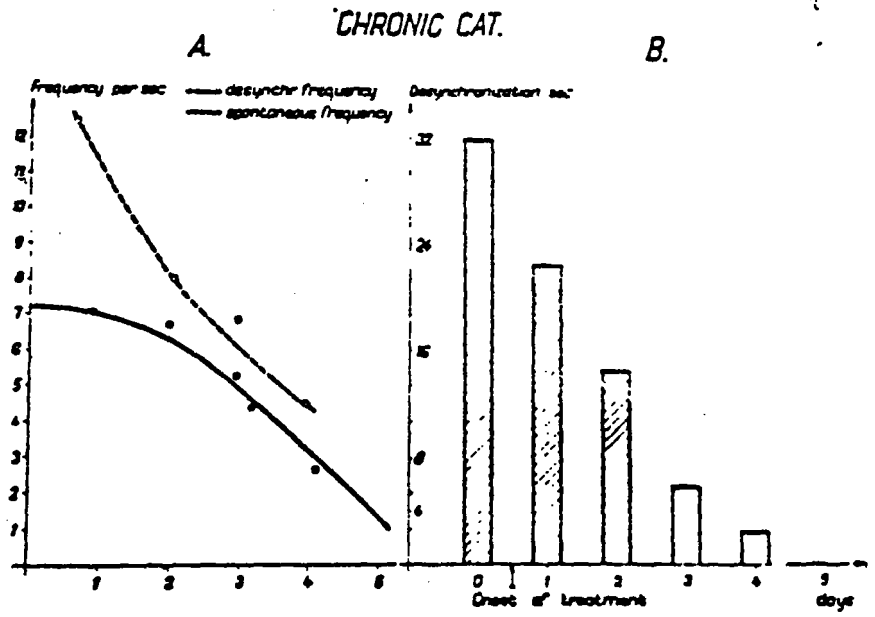


Fig. 4.—*A*, slowing of spontaneous and desynchronization frequency in chronic experiment; abscissa is number of days; ordinata is frequency per second. *B*, decrease in the duration of desynchronization. On the fifth day no desynchronization is shown at all. Abscissa shows number of days. Ordinata shows duration of desynchronization in seconds. Arrow marks onset of treatment.

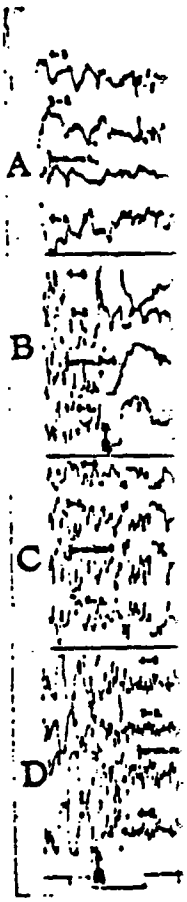


Fig. 6.—

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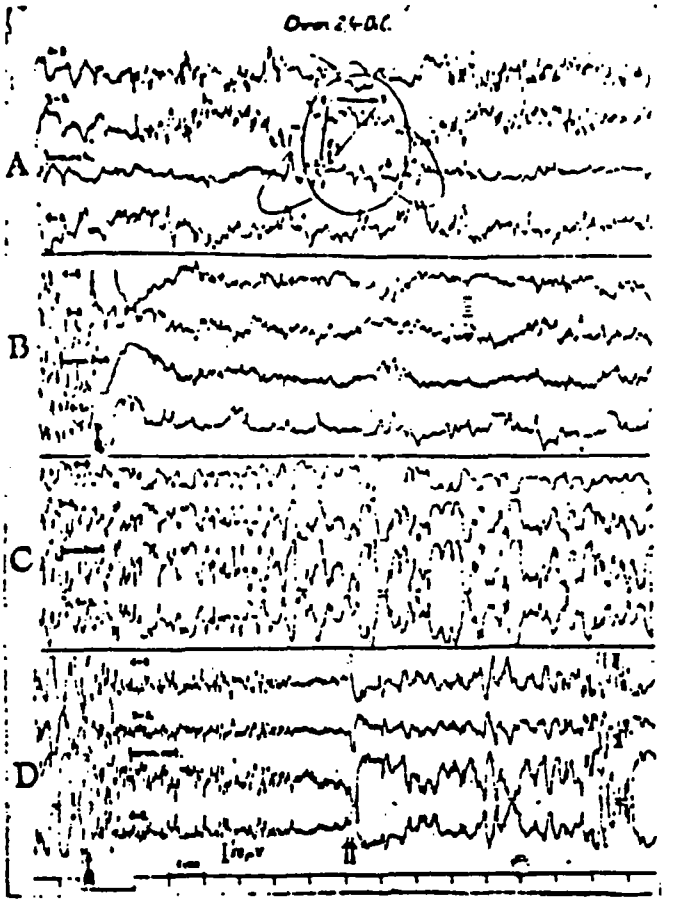


Fig. 5.—Electroencephalogram of cat subjected to chronic treatment. Designations are as in the former cases. Arrow marks sound stimulus. *A*, spontaneous activity prior to treatment, normal waves of 7-per-second frequency. *B*, desynchronization manifesting itself due to sound stimulus, prior to treatment; duration: 32 sec. The entire duration cannot be marked on the diagram. Frequency is 12.5 per second. *C*, spontaneous activity on the fourth day of treatment; large, slow waves of 2-per-second frequency. *D*, desynchronization due to sound stimulus on the fourth day of treatment, significant shortening in duration. Frequency is 6 per second. Double arrow marks end of desynchronization.

CONDITIONED REFLEXES

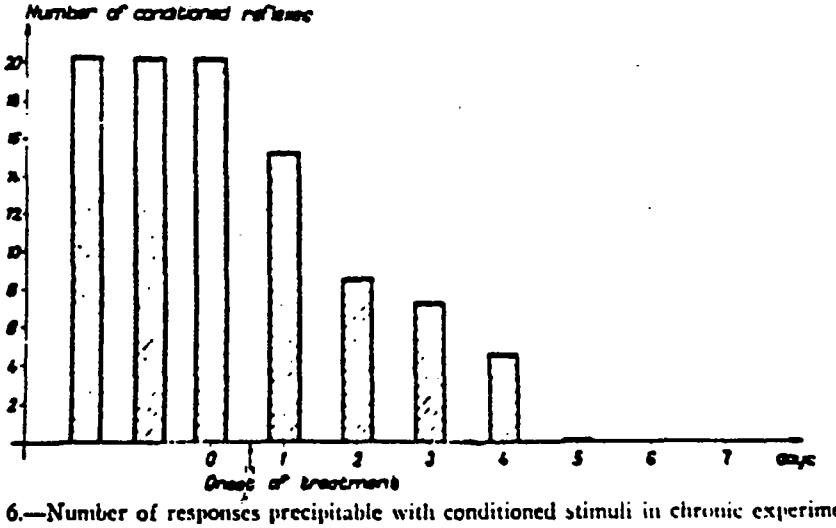


Fig. 6.—Number of responses precipitable with conditioned stimuli in chronic experiment.

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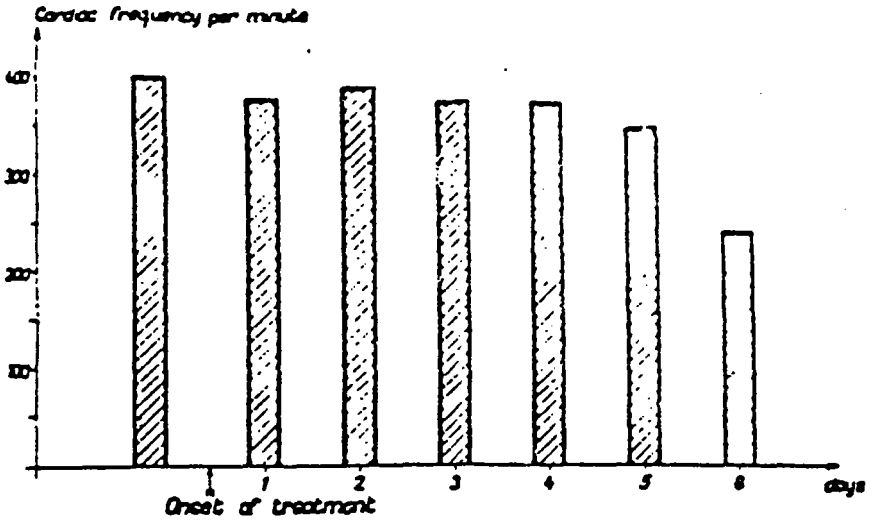


Fig. 7.—Change of rat cardiac frequency in chronic experiment.

In the second part of the experiments, 8 rats were examined in relation to the effect of 2,4-D on higher nervous activity, using a conditioned-anxiety-reflex method.

The response to conditioned stimuli of experimental animals under continuous treatment with 2,4-D was decreased day by day despite reinforcements given with the unconditioned stimulus every day. The decrease in the number of conditioned-reflex responses was 21% by 24 hr., 58% by the second day, 63% by the third day, and 79% by the fourth day. By the fifth day, no conditioned-reflex response could be elicited at all (Fig. 6).

The rats in the chronic experiments had daily determinations of cardiac output. It decreased by 6% on the first day and 35.5% by the sixth day (Fig. 7).

Other symptoms manifesting themselves were found to correspond to those mentioned in the literature. These alterations, however, were seen only on the third or fourth day of treatment. The average weight loss of the



Fig. 8.—Dorsal segment of the spinal marrow of rat subjected to chronic treatment (high magnification); demyelinization detectable.

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rats was 23% in the course of the experiment. The animals generally died on the sixth day.

Histology failed to reveal changes in the grey or white matter of the brain of the animals. A myelopathy manifested as demyelinization was detectable in the dorsal portion of the spinal cord. With Spielmeyer's staining method the nerve fibers showed demyelinization partly in the pyramidal tract and partly in the Goll and Burdach bundles (Fig. 8).

Comment

In evaluating these experiments it is necessary to separate the EEG changes when 2,4-D was administered intraperitoneally from those occurring after direct cortical application. The latter series of experiments were performed in order to ascertain whether 2,4-D itself or some of the metabolized products formed in the organism caused the EEG changes.

2,4-D crystals placed on the cortex brought about a reversible increase in frequency and decrease in amplitude obvious in the course of excitation of the cortical cells. Tyrosine, which is very close to 2,4-D chemically, did not cause any change whatever. Thus the action must be considered specific. Since 2,4-D also produced changes when placed directly on the cortex, it is very likely that the 2,4-D molecule itself, and not some of its decomposition products, precipitated the pathological symptoms after the drug's introduction into the organism.

2,4-D administered intraperitoneally exerted an effect of a different type. In the acute EEG experiments it reversibly abolished the normal desynchronization of the cortex and of the reticular formation. Since this blocking began on the average of 10 min. after the injection and was over in 60 min., it may be assumed that the effect was due to 2,4-D on cerebral cells via the blood stream, paralyzing normal function. Later, with a decrease in the concentration of 2,4-D, normal function was restored.

Even on the first and second day after onset of the treatment there was a slowing in frquency and a marked decrease in the duration of desynchronization in the chronic EEG experiments. By the fourth day the characteristic large, slow, toxic waves appeared on the electroencephalogram. On the fifth day no desynchronization manifested itself due to the constantly increasing toxicosis. On the basis of all these findings, it is very likely that 2,4-D accumulates in the organism and in the cerebral tissue and causes a constantly more severe paralysis in the function of the latter.

From the conditioned-reflex examinations it is obvious that 2,4-D also damages the higher nervous activity. This effect takes place as early as 24 hr. after exposure and by 48 hr. is rather pronounced. By the fifth day all the conditioned reflexes cease.

It was impossible to perform conditioned-reflex and EEG studies in one and the same animal. However, the results obtained in the 2 groups of animals are parallel. After 24 hr. there was a 34.2% decrease in the duration of desynchronization and a 21% decrease in the conditioned-reflex responses. On the fifth day there is no desynchronization in response to stimulation, and the conditioned reflexes cease completely.

The question of the point of attack may now be raised. It is known that the intactness of not only the cortex but also of the reticular formation in the mesencephalon is necessary for both normal desynchronization and conditioned-reflex responses.

Electroencephalograms showed the same changes both in the cortex and in the reticular formation. Separate changes referring only to lesions of the cerebral cortex were not demonstrable. Desynchronization and conditioned-reflex responses decreased and disappeared simultaneously. These data permit the assumption that intraperitoneal administration of 2,4-D primarily damages the reticular formation. Then, the lesions induced in this region paralyze the function of the cerebral cortex.

Histology failed to demonstrate any changes either in the cortex or in the sub-

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cortical regions, allowing the conclusion that no obvious morphological change is produced in the nerve cells by 2,4-D. Obviously the metabolism and biological functions of the nerve cells are damaged by 2,4-D. The possibility of submicroscopic changes in the structure cannot be excluded.

The histological changes manifesting themselves in the region of the spinal cord may be responsible for the paralyses of the extremities.

On the basis of the data obtained in animal experiments it must be assumed that human subjects exposed to the action of 2,4-D also may suffer via smaller dose, milder disturbances in function of the nervous system and of higher nervous activity respectively.

Functional changes in the nervous system demonstrated themselves after 24 hr., other pathologic symptoms becoming obvious only on the third or fourth experimental day. It is possible that, in laborers working with 2,4-D in whom routine methods fail to demonstrate pathologic symptoms, EEG studies may call attention to early changes. It is desirable therefore that workmen engaged in spraying 2,4-D be protected from exposure to the poisonous drug and that increased attention be paid to the detection of early signs of toxicity.

Summary

After the parenteral administration of 2,4-D a reversible inhibition of cerebral electrical activity was observed in the acute experiments, and in chronic experiments the same was present to a gradually increasing degree. Toxic EEG signs were developing. According to conditioned-reflex experiments, the higher nervous activity suffered severe damage. The changes are probably produced by the 2,4-D molecule itself and not by some of its degeneration products. The point of attack seems to be the reticular formation. The changes manifest themselves as early as 24 hr. after exposure.

The changes found in animal experiments suggest the need of caution in the use of 2,4-D. Increased protection and special neurological examination of workmen in contact with 2,4-D is necessary.

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Reprint from the JOURNAL of the American Veterinary Medical Association, Vol. 143, No. 4, Aug. 15, 1963, pp. 398-399.

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Chronic Toxicity of 2,4-D Alkanolamine Salts to Cattle

J. S. Palmer, D.V.M., M.P.H.

AVAILABLE DATA indicate the low toxicity of chlorinated phenoxyacetic acid compounds to cattle.[1,3-6] These conclusions are based on results derived from direct oral dosage of cattle or from exposing cattle to pastures sprayed with various herbicidal concentrations. The hazard is negligible when herbicides are applied in concentrations that effectively control dicotyledonous plants.

Many different herbicidal compounds have been introduced on the market, but 2,4-D was the first of its general type to be accepted. It has been used in vast amounts for controlling annual weeds in cereal crops and pastures and also for weeds and brush in turf and ranges. Research data on other animal species indicate that it is relatively more toxic than other compounds.[2]

The likelihood of cattle gaining access to intoxicating amounts of this herbicide in sprayed areas, spray tanks, and storage containers prompted this study.

Materials and Methods

Of 6 yearling steers weighing 300 to 400 lb. used initially, 5 were Holstein-Friesians, and 1 was a Hereford cross. The steers were fed a maintenance ration consisting of a protein concentrate each morning and a combination of hegari and alfalfa hay each evening. The ration was increased when weight loss was detected in the principals as well as in the control. Weights were determined weekly.

The 2,4-D test herbicide contained 67% alkanolamine salt.* The daily dose, on a 5-day-per-week regimen, was calculated at a rate of 50, 100, 200, and 250 mg./kg. of body weight. The daily dose of the liquid herbicide varied from 12.5 to 70.5 Gm. and was administered with a dose syringe, using water as the vehicle. Each steer was restrained in a squeeze chute to assure accurate dosing.

One steer each was treated with 50, 100, and 200 mg./kg. and 2 steers with 250 mg./kg. An untreated steer was maintained as a control.

Results

One steer treated initially with 70.46 Gm. (250 mg./kg.) was eliminated from the study. A chronic respiratory infection developed, considered as incubating before the test with the focal point in the pharyngeal region. The herbicide treatment probably acted as an irritating factor and set up a fulminating condition. The characteristic signs of labored respiration and weight loss were observed. The steer was treated after 4 weeks with appropriate therapy and then sold after regaining weight. Also, pneumonia unexpectedly developed in the control steer, but antibiotic therapy brought quick recovery.

A direct relationship was noticed between the quantity of daily intake, the accumulated total, and the length of the treatment period. With the increase in daily dose, less time was required to produce signs of poisoning so that the total amount ingested was decreased (Table 1).

The steer treated with 50 mg./kg. was given 112 daily treatments (5 per week) ranging from 12.5 to 19.7 Gm. During this time, the steer's weight increased from 358 to 563 lb. The treatment period extended from June 25 to Dec. 4, 1962, with an approximate average of 4.5 treatments weekly. When the study ended, this

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The writer thanks the laboratory employees W. Rector, T. Secor, and H. Jobes in initiating this study.

*The test herbicide was a commercial product, labelled "2,4-Dow Weed Killer, Formula 40," produced by the Dow Chemical Co., Midland, Mich.

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TABLE 1—Chronic Toxicity of 2,4-D Alkanolamine Salt to Cattle—Relationship of Daily Dose to Accumulated Dosage

Steer No.	Daily dose		No. treatments*		Accumulated dosage (Gm.)		Results
	Basis (mg./kg.)	Variation (Gm.)	To produce intoxication	Total	To produce intoxication	Total	
212	50	12.5-19.7	—	112	—	1829.3	Unaffected
213	100	25.9-35.7	86	88	2546.4	2616.0	Poisoned
214	CONTROL						
215	250	70.5	—	1	—	70.5	Disqualified
216	200	54.2-58.7	34	44	1419.6	1958.6	Poisoned
219	250	51.8-53.3	15	20	784.5	1049.1	Poisoned

*Given daily 5 days a week.

steer had no apparent signs of intoxication.

Cattle given doses of 100, 200, and 250 mg./kg. had signs of poisoning after 86, 34, and 15 treatment days, respectively. The more heavily dosed steers were treated to termination of the experiment. The steer given 100 mg./kg. had to be medicated after it developed a digestive disorder characterized by extreme tympany. Two days were required to correct the condition, although anorexia was never observed. Rumen atony from accumulated herbicide was apparently the predisposing factor; the steer was removed from the experiment and made an uncomplicated recovery.

Primary signs of intoxication in steers given 200 and 250 mg./kg. of the compound were development of dry cracked muzzles and ulcerated mucous membranes, with epistaxis on routine restraint. A clear, viscid nasal discharge, associated with a fetid odor, was noticed. Progressive apathy and depression were apparent.

One steer given 250 mg./kg. had moderate tympany associated with melena. Necropsy revealed severe hemorrhages in the large intestine and pulmonary congestion. The steer given 200 mg./kg. had muscular weakness in the hindquarters and a staggering gait. Gross pathologic changes noticed at necropsy were inflammatory swelling of the kidneys and a pneumonic condition considered to be the immediate cause of death.

Summary and Conclusions

Daily oral doses of alkanolamine salts of 2,4-dichlorophenoxyacetic acid (2,4-D) were given to 5 yearling steers to determine the chronic toxicity of this compound. The doses were based on milligrams of 2,4-D per kilogram of body weight and were administered by dose syringe, with water as the vehicle. Doses were given daily 5 days a week.

Signs of poisoning were noticed in the more heavily dosed steers (250 mg./kg.) after 15 treatments, in contrast to 86 treatments at 100 mg./kg. A dose of 50 mg./kg. had no apparent effect on 1 steer after 112 doses. A direct relationship was thus detected between the relative quantity of daily intake to the accumulated total dosage and the length of the treatment period.

It was concluded that this herbicide has a low degree of chronic toxicity, apparently because it is not readily stored in bovine tissues. However, it is possible for cattle to ingest enough to produce morbidity and mortality.

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Toxicity of 2,4-Dichlorophenoxyacetic Acid for Man and Dog

JOHN H. SEABURY, MD
NEW ORLEANS

In November, 1959, Goldstein, Jones, and Brown [1] reported three cases of peripheral neuropathy following exposure to an ester of dichlorophenoxyacetic acid (2,4-D). The first patient had spilled 60 ml of a 10% solution of an ester of 2,4-D on his forearms without washing it off. The patient felt unduly fatigued on the evening of the same day, and within the next ten days he developed nausea, vomiting, and 20-pound weight loss. Approximately two months later he had a similar exposure on his legs, and he developed nausea, vomiting, and diarrhea during the subsequent five days. One week after the second exposure, he complained of numbness and aching of the digits of all extremities. There was desquamation of the skin of the palms and soles, and within six weeks after the second exposure he had well-developed neuropathy.

The second patient had a less determinable amount of exposure by dermal wetting with an ester of 2,4-D. Shortly after exposure, she noted swelling and aching of the feet and legs, which persisted for approximately 2½ weeks. Approximately one year later she had a similar exposure to the herbicide with recurrence of similar symptoms. However, she later developed anorexia, loss of 20 pounds in weight, and severe pain in her legs with painful swelling of the metacarpal joints of both hands. Approximately five months after her second exposure she had a flaccid paraparesis.

The third patient reported had been spraying with a solution of an ester of 2,4-D. During this activity his sleeves and trouser legs had been wetted with the herbicide, and some of the spray might have been inhaled. On the following day, he noted malaise, headache, nausea, and vomiting. Vertigo was severe on the second day following spraying. Paresthesias in the extremities and pain in the legs appeared within four or five days, followed by twitching of the muscles in the calves and arms. Fasciculations became generalized. There were no neurological or

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electromyographic changes, but his symptoms persisted.

In a subsequent comment [2] on this report of peripheral neuropathy following exposure to an ester of 2,4-D, Goldwater points out that these are the first such cases to be reported and that one would have to know the composition of the vehicles, solvents, and emulsifiers used in the commercial preparation before incriminating 2,4-D as the principal toxic compound. Goldstein et al agreed with Goldwater's comment and stated that in one instance they were able to obtain the label from the container of the 2,4-D preparation. On this label, the preparation was stated to contain "2,4-dichlorophenoxyacetic acid, isopropyl ester 44% and inert ingredients 56%."

Because of these reported cases of toxicity to 2,4-D, and the recent study of a man claiming disability of a different type subsequent to spraying water hyacinths with a dichlorophenoxyacetic compound, I decided to report certain therapeutic trials with plant hormones carried out in 1948 and 1949.

My interests in plant hormones were therapeutic rather than toxicologic, and since no therapeutic efficacy was demonstrated in the trials, no attempt was made to publish the results. In 1948-1949, there was no known therapeutic agent for disseminated coccidioidomycosis, histoplasmosis, or cryptococcosis. It appeared remotely possible that the plant hormones might alter the course of these fungous infections. Since we were unable to find any evidence of therapeutic effectiveness in the literature, and only meager reports of animal toxicity, I first screened a mixture of indole-3-acetic acid, indole-3-propionic acid, α-naphthaleneacetic acid, and 2,4-dichlorophenoxyacetic acid in guinea pigs infected with *Coccidioides immitis*. No therapeutic effect was noted.

Six female mongrel dogs, of approximately one year of age, were infected with *Histoplasma capsulatum*. Three dogs were kept as controls, and three were treated parenterally with the sodium salt of 2,4-dichlorophenoxyacetic acid. Only the treated dogs are of interest here. Dog 1 received a total of 464 mg of sodium 2,4-D in 28 injections over 37 days, of which 288 mg were given intravenously. On a weight basis, the injections amounted to 1.17 mg/kg of body weight. Dog 2 was given 389 mg in 24 injections over 32 days, of which 261 mg were given intravenously. On a weight basis, this amounted to 3.2 mg/kg of body weight per injection. Dog 3 received 389 mg in 24 injections over 32 days, of which 277 mg were given intravenously. The dose per injection was 2.6 mg/kg of body weight. Dog 1 was autopsied 202 days after the first injection of 2,4-D; dog 2 was autopsied on the 86th day following the initiation of treatment; and dog 3 was autopsied on the 85th day. All dogs had gained substantial weight during the experimental period. All dogs showed evidence of visceral histoplasmosis at autopsy, but there were no gross or microscopic lesions in the liver, spleen, lungs, kidneys, or adrenals other than those due to histoplasmosis. No neurological tissues were examined.

A moribund patient with disseminated coccidioidomycosis was selected for intramuscular injection of auxins. This patient received a total of 40 mg of the sodium salt of 2,4-D, 3.3 mg of indole-3-propionic acid, 3.3 mg of indole-3-butyric acid, and 3.3 mg of α-napthaleneacetic acid during a period of four days. Death occurred on the fifth day. The patient exhibited no unusual symptoms during treatment, and the autopsy findings were only those of generalized coccidioidomycosis and hypoxia.

In April, 1949, I decided to treat a 48-year-old male patient with disseminated coccidioidomycosis with auxins to the limit of tolerance. It was obvious that the patient would die within the next several weeks unless the course of the disease could be modified. The details of treatment appear in Table 1.

Intravenous infusion of 2 gm of 2,4-D produced no changes in his physical examination and provoked no complaints from the patient. When 3,600 mg of 2,4-D were given intravenously during a period of two hours, a reaction appeared. During the latter part of the infusion, the patient became very quiet, semistuporous, and exhibited fibrillary

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TABLE 1.—*Administration of Auxins to Man*

Date	Route of Administration	2,4-D, Mg	Indole-3-Butyric Acid, Mg	α-Naphthalene-acetic Acid, Mg	Reaction
3/ 4/49	IM	8	0.66	0.66	Local burning
3/ 5/49	IM	24	1.98	1.98	Procaine added—none
3/ 6/49	IM	16	1.32	1.32	"
3/ 7/49	IV	160	6.6	6.94	None
3/ 9/49	IM	24	1.98	1.98	None
3/10/49	IV	120	13.2	13.2	None
3/11/49	IV	120	13.2	13.2	None
3/15/49	IV	240			None
3/16/49	IV	480			None
3/18/49	IV	400			None
3/19/49	IV	960			None
3/21/49	IV	960			None
3/23/49	IV	800	33		None
3/24/49	IV	800	33		None
3/25/49	IV	800	33		None
3/26/49	IV	800	33		None
3/28/49	IV	800	33		None
3/30/49	IV	960	33		None
4/ 1/49	IV	800	33		None
4/ 2/49	IV	800	33		None
4/ 4/49	IV	800	33		None
4/ 6/49	IV	2,000	33		None
4/ 8/49	IV	3,600			See text

The 2, 4-D was administered as the sodium salt. All intravenous medications were administered in 1,000 ml of 5 % dextrose in water

movements about his mouth and in both hands and forearms. The fibrillary twitchings persisted for several hours. Stupor deepened to the point where he could not be roused by sound but did respond to painful stimuli. Stupor was accompanied by hyporeflexia at the knees, ankles, and biceps tendons. Hyporeflexia persisted for 24 hours. Urinary incontinence appeared and persisted for 24 hours. There was no change in pulse rate or volume, or respiratory rate or depth. Seven hours after the termination of the infusion, the patient could be aroused verbally but quickly lapsed into what ap-

TABLE 2.—*Acute Toxicity of 2,4-D for Animals*

Animal and No. Used	Route of Admin.	Dose-Toxicity, Mg/Kg		Ref. No.
Mouse				
Approximately 450	Stomach tube	LD_{50}*	375	4
	Intraperitoneal	LD_{50}*	375	4
Number not stated	Subcutaneous	LD_{50}*	250	3
Rat	Stomach tube	LD_{50}*	666	4
Approximately 150	Intraperitoneal	LD_{50}*	666	4
Guinea pig	Stomach tube	LD_{50}*	1,000	4
Approximately 125	Intraperitoneal	LD_{50}*	666	4
Rabbit	Intraperitoneal	LD_{50}*	400	4
Approximately 70	Intravenous	LD_{50}*	400	4
	Stomach tube	LD_{50}*	800	4
Dog	Oral	LD_{50}	100	5
4 at this dosage				
2	Oral	LD_0†	500	5
Monkey	Intraperitoneal	LD_0*	425	4
1				
Chick	Oral	LD_0‡	350	6
5 in each group		LD_{100}‡	700	6

* Administered as the sodium salt of 2,4-D.
† Given as butyl ester. All figures calculated as 2,4-D.
‡ Given as an alkanolamine of 2,4-D.

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TABLE 3.—*Subacute Toxicity of 2,4-D for Animals*

Animal and No. Used	Route of Admin.	Dose-Toxicity, Mg/Kg/Da	Ref No.
Dogs	Intravenously		
2		200 × 2 LD_{100}	4
2		100 × 2 LD_{100}	4
3		50 × 6 (1 died, 1 sacrificed very ill)	4
1		25 × 6 (sacrificed very ill)	4

peared to be deep sleep. When he was aroused, there appeared to be lack of recognition of familiar visitors and apathy. Eighteen hours after receiving the hormone, he talked spontaneously and recognized everyone. Twenty-four hours after the infusion he was oriented, eating well, but still lethargic and complaining of profound muscular weakness. Psychomotor retardation was evident. Within 48 hours he had returned to his prereaction status insofar as could be observed. There was no further evidence of neurologic or muscular change in the subsequent two weeks of his life.

His maximum temperature on April 6, 1949, was 103.8 F (39.9 C) and was 104.4 (40.2 C) on April 7. For 48 hours after the intravenous infusion of 3.6 gm of 2,4-D his temperature did not exceed 101 F (38.3 C). He did not have his usual daily rigor. His temperature remained between 102.2 and 102.6 F (39.0 to 39.2 C) maximum for six more days and then resumed its previous level of approximately 104 F (40.0 C) daily.

On the night of his reaction, the spinal fluid was under normal pressure, contained no cells, and the glucose and chloride concentrations were normal. Unfortunately, the protein content was not determined. Approximately 14 hours after the last infusion of 2,4-D, the blood urea nitrogen was 56 mg%, the fasting blood glucose 85 mg%, and the carbon dioxide combining power 48 vol%. The blood urea nitrogen was 12 mg% on April 12, 1949.

There was no alteration in the clinical course of the patient following discontinuation of treatment. The patient died 17 days after the last infusion of 2,4-D.

Autopsy was performed 4½ hours after death. The gross and microscopic findings were typical of disseminated coccidioidomycosis and cachexia.

The heart, pancreas, adrenals, and striated muscle taken from the diaphragm were normal grossly and microscopically.

The liver weighed 1,720 gm and was light brown in color. Congestion was present. Microscopically, the lobular pattern was well preserved. The central veins and their radiating sinusoids were slightly congested. Throughout the parenchyma were numerous focal areas of necrosis which were composed primarily of a granulomatous tissue infiltrated by lymphocytes and polymorphonuclear leukocytes. Several giant cells were seen in some of the focal

TABLE 4.—*Chronic Toxicity of 2,4-D for Animals*

Animal and No. Used	Route of Admin.	Dose-Toxicity	Ref No.
Rat	In feed		
7		100 mg/kg of feed/da × 21, then 1,000 mg/kg of feed/da × 10—no effects	4
7		200 mg/kg of feed/da × 30—no effects	4
7		400 mg/kg of feed/da × 30—no effects	4
5 or 6	Oral (intubation)	3 mg/kg 5 ×/wk × 4 wk—no effects	9
5 or 6		10 mg/kg 5 ×/wk × 4 wk—no effects	9
5 or 6		30 mg/kg 5 ×/wk × 4 wk—no effects	9
5 or 6		100 mg/kg 5 ×/wk × 4 wk—depressed growth, gastrointestinal irritation, cloudy swelling of liver	9
5 or 6		300 mg/kg 5 ×/wk × 4 wk—LD_{100}	9
Dog	Oral		
2		2 mg/kg/da × 90—LD_0	5
2		5 mg/kg/da × 90—LD_0	5
3		10 mg/kg/da × 90—LD_0	5
4		20 mg/kg/da—LD_{75} (da 18, da 25, da 49)	5
Mice Number not stated	Parenteral	1/3-1/5 LD_{50}/da × 21-90—LD_0 * ¼ LD_{50}/da retards growth	3
Chicks 5 in each group	Oral	0.25 mg/kg 3 × wk × 4 wk 2.5 mg/kg 3 × wk × 4 wk 25 mg/kg 3 × wk × 4 wk 250 mg/kg 3 × wk × 4 wk (250 mg/kg produces slight retardation of growth)	LD_0 6

* Mice receiving one-third LD_{50} per day have become pregnant and borne apparently normal

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granulomas, and a rare *Coccidioides immitis* could be found within the giant cells. These changes were considered due to coccidioidomycosis.

The right kidney weighed 180 gm and left kidney 170 gm. The capsule stripped with ease from each kidney. Cortical and medullary striations were distinct. Except for marked congestion of the capillary bed throughout, especially at the periphery of the cortex and in the glomeruli, there were no microscopic changes other than those due to numerous granulomatous lesions containing *Coccidioides immitis*.

The brain weighed 1,270 gm. The entire brain and coverings were normal grossly except for the leptomeninges over the brain stem which were opaque. A pressure cone was seen involving the cerebellum. Multiple sections from the cerebral hemispheres, brain stem, and cerebellum were examined. No microscopic abnormalities were detected.

Comment

The acute, subacute, and chronic toxicity of 2,4-D for laboratory animals is summarized in Tables 2, 3, and 4. An extensive tabulation of the oral toxicity of the 2,4-D and 2,4,5-T type herbicides manufactured by the Dow Chemical Company appears in the article by Rowe and Hymas,[9] and this article should be consulted by those interested in possible toxicity to livestock.

Two monkeys were given 2,4-D intraperitoneally,[4] but only the larger dose is recorded in Table 2.

When guinea pigs were exposed to either a wet cloud of the sodium salt of 2,4-D in a spraying chamber or to a dry cloud of the crude acid, none of the animals exhibited any symptoms and there was no gross evidence of lung irritation.[4]

The symptoms of acute toxicity in mice, rabbits, guinea pigs, and rats are essentially the same.[3,4] Some animals die suddenly, apparently from ventricular fibrillation. Those that do not die suddenly develop stiffness of the extremities, incoordination, lethargy, stupor, coma, and death. True myotonia occurs and is similar to that produced by cevadine (veratrine).[3,7] Hill and Carlisle[4] state that the lower extremities become paralyzed, and all authors agree that myotonia is more evident in the lower extremities than in the upper.

Dogs exhibit the same general symptoms with marked spasm of the hind limbs. In addition, they may show sneezing, rubbing of the eyes, diarrhea, and vomiting. Those living long enough refused all food.[3,5]

Two monkeys given 1 gm and 1.5 gm of sodium 2,4-D, respectively, by the intraperitoneal route exhibited nausea and vomiting initially. The gastrointestinal symptoms were followed by stiffness of the legs, with some muscular incoordination, lethargy, hanging of the head, and ptosis of the eyelids.[4] The symptoms first appeared about four hours after injection and persisted for about 48 hours before lessening in intensity. At the end of five days, recovery was complete.

The patient reported here had received 12,712 mg of sodium 2,4-D parenterally prior to the intravenous dose which provoked a reaction. Consequently, one cannot state that the changes produced by 3,600 mg of sodium 2,4-D intravenously were entirely related to this single dose. Despite this, there was a considerable similarity between the reaction produced in the human patient and that produced in the two monkeys. However, on a weight basis, the dose administered to our patient intravenously was approximately 66 mg/kg of body weight whereas the maximum intraperitoneal dose given to the monkeys was 428 mg/kg of body weight.

Although the majority of studies of acute toxicity of the 2,4-dichlorophenoxyacetic compounds are in close agreement, the number of animals tested by some authors was small, and some disagreements appear which cannot be resolved on the basis of the data presented. For example, the acute LD_{50} for oral administration of 2,4-D to dogs was 100 mg/kg according to Drill and Hiratzka[5] based on the study of four animals. Other workers[8] gave an amount of the butyl ester of 2,4-D calculated to contain 500 mg of 2,4-D per kilogram to each of two dogs without observing any deleterious effects within 96 hours. One of the two dogs was autopsied and showed no evidence of any macroscopic lesions. The other animal remained in apparently excellent health for the following

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82 days. One would hesitate to conclude from these data that the butyl ester is less toxic than 2,4-dichlorophenoxyacetic acid.

The lowest dosage administered intravenously to dogs (25 mg/kg) over a period of six days was toxic,[4] producing decubitus ulcers of the extremities and necrotizing lesions about the mouth. A lower limit for subacute toxicity in dogs has not been established.

According to Drill and Hiratzka[5] the largest dose of 2,4-D which could be given orally to dogs for extended periods was 10 mg/kg of body weight.

Commercial herbicidal preparations of the chlorinated phenoxyacetic acids and associated compounds are usually formulations of one or more active ingredients with solvents, dispersing and wetting agents, and sometimes co-solvents. These may contain as active ingredients either the acids or their salts, esters, or amines. The exact composition of liquid formulations is often not stated in labeling. Although Rowe and Hymas[9] stated that the acute oral toxicity of the commercial formulations of the Dow Chemical Company tended to be proportional to that expected from their content of active ingredients and that the "inert ingredients" did not appear to exert a potentiating effect upon toxicity, Hill and Carlisle[4] found that the toxicity of 2,4-dichlorophenoxyacetic acid dissolved in tributylphosphate and oil was much greater than the toxicity of 2,4-D alone. The increase in toxicity was due to the tributylphosphate-oil mixture itself. The same authors performed similar studies with N-butyl alcohol-oil solutions of 2,4-D, and stated "Although not conclusive, these experiments indicated that N-butyl alcohol-oil concentrations of 2,4-D are at least of the same order of toxicity as the tributylphosphate-oil solutions." These findings by Hill and Carlisle lend strength to Goldwater's criticism[2] of the report by Goldstein.[1]

Bucher,[3] without indicating the number of animals studied, stated that the histopathology produced by 2,4-D in acutely intoxicated mice consisted of wide dilatation of the blood vessels of lungs, liver, and kidneys. She noted moderate atrophy of the liver in one chronically treated dog. There was no significant variation in the peripheral blood of chronically treated mice from that observed in controls with respect to hemoglobin, red and white blood cell concentrations, and differential counts.

Hill and Carlisle[4] found that rats and guinea pigs dying of massive doses of 2,4-D consistently exhibited congestion of the viscera and enlarged swollen kidneys. Microscopically, there was massive cloudy swelling of the proximal convoluted tubules with cast formation. The glomeruli and blood vessels were unaffected. Occasional animals showed slight patchy pulmonary edema and alveolar hemorrhages. Rabbits that received injections of one fourth of the LD_{50} parenterally for from 4 to 13 days showed slight-to-moderate degenerative changes in the proximal convoluted tubules. Scattered petechial hemorrhages were present in the lungs of a few animals. Three to seven daily injections of one half of the LD_{50} produced a significant decrease in hemoglobin in four of six rabbits. The renal changes were similar to those observed in the rabbits receiving one quarter of an LD_{50} for a longer period of time.

In addition to the development of bleeding and necrosis of the gums and decubitus ulcers, dogs also exhibit evidence of liver damage more frequently than other animals studied.[3-5] The histopathology varied from occasional areas of focal necrosis in animals subjected to chronic oral toxicity studies to extensive necrobiosis throughout the liver in one dog[4] given two daily injections of 200 mg/kg of the sodium salt of 2,4-D. For the most part, dogs succumbing to massive doses of 2,4-D showed centrolobular degeneration, atrophy and lysis of the parenchymal cells about the central veins of the lobules, and congestion and dilatation of the paracentral sinusoids.[4] Dogs also showed cloudy swelling of the renal tubular epithelium,[4,5] and one dog receiving a massive dose[4] showed actual tubular epithelial necrosis. Lymphoid necrosis was observed in the acutely poisoned

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dogs, and some of them showed terminal lymphopenia in the peripheral blood.

References to human toxicity are meager. Hildebrand [10] made the simple statement "Some investigators have consumed the chemical and suffered no ill effects." Assouly [11] stated that workers employed in the fabrication of an ester of 2,4-dichlorophenoxyacetic acid developed symptoms of somnolence, anorexia and gastralgia, increased salivation, a sweet taste in the mouth, a sensation of drunkenness, heaviness of the legs, and hyperacusis. Whether these symptoms were related to the 2,4-dichlorophenoxyacetic acid or to the chemicals used in the process of esterification is not known. Certainly a sweet taste in the mouth with sialarrhea suggests the symptoms were related to the chemicals used in esterification. Hyperacusis was not noted in either of the two patients reported here who received parenteral 2,4-D. Mitchell, Hodgson, and Gaetjens [12] state in a footnote "Dr. E. J. Kraus of the University of Chicago reported on January 26, 1946, that a human (adult male) had taken daily by mouth, 300 mgm. of purified 2,4-D either after the midday or evening meals during a period of 21 days without experiencing ill effects."

It would seem logical that chemical modification of the chlorinated phenoxyacetic acids might well result in altered pharmacological action and toxicity. An interesting example of this is the dimethylaminoethyl ester of *p*-chlorophenoxyacetic acid. This ester was produced by Thuillier, Rumpf, and Thuillier.[13] The ester has an LD_{50} for mice of 330 mg/kg when given intravenously and an LD_{50} of 845 mg/kg when given intraperitoneally to dogs. Chronic toxicity studies in rats indicated that 300 mg/kg could be given for more than five months without alteration of the blood cell count or of the organ systems. However, it appeared to act as a central nervous system stimulant and was capable of raising a lowered arterial blood pressure. There was no evidence of a myotonic action. Coirault, Pourpre, Damasio, Rouif, Deligne, David, and Talairach [14] reported using this ester therapeutically on the neurosurgical service of two hospitals in the management of stuporous and comatose patients. The oral dose for man averaged 400 to 1,200 mg in 24 hours, but doses of 4 gm have been given without reaction. The ester was given intravenously in doses of 250 to 500 mg at the rate of about 250 mg in five minutes. The authors state that this material had been given for from eight days to six months without a single reaction. No information is given concerning the possible herbicidal activity of this chlorophenoxyacetic acid ester.

Summary

Three dogs with experimental histoplasmosis were screened for chronic parenteral toxicity of sodium 2,4-dichlorophenoxyacetic acid. The dogs were given parenteral sodium 2,4-D at the rate of 1.17 mg/kg of body weight, 3.2 mg/kg of body weight, and 2.6 mg/kg of body weight per injection. Treatment extended between 32 and 37 days. In this dosage, there was no evidence of chronic toxicity.

One patient was given a total of 40 mg of the sodium salt of 2,4-D, 3.3 mg of indole-3-propionic acid, 3.3 mg of indole-3-butyric acid, and 3.3 mg of α-naphthaleneacetic acid intramuscularly during a period of four days. No toxicity was observed.

A second patient was given a total of 369 mg of indole-3-butyric acid, 38.3 mg of α-naphthaleneacetic acid, and 12,712 mg of the sodium salt of 2,4-dichlorophenoxyacetic acid parenterally during a period of 34 days without observable toxicity. Most of this material was given intravenously. Two days later, 3,600 mg of sodium 2,4-D were given intravenously and produced definite clinical signs of toxicity. The patient recovered clinically from this toxic episode within 48 hours, and showed no further evidence of neurologic or muscular change in the subsequent two weeks of life. Death was due to disseminated coccidioidomycosis. The autopsy findings are described.

A single intravenous dose of 2,000 mg of sodium 2,4-D did not produce clinical evi-

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dence of toxicity in the second patient, whereas 3,600 mg was definitely toxic.

A total of 16,312 mg of sodium 2,4-D was administered to the second patient. There were no necropsy findings which could be attributed to toxicity of 2,4-dichlorophenoxyacetic acid.

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In countries where public health has been longest in effective operation, and where natality has responded to the new pattern of living, the demographic structure has undergone profound change, with a shift of population to older groups. Thus over-population and ageing are new challenges no less demanding than some of the infectious diseases which are passing. Moreover, where public health has succeeded emphasis has shifted from infectious to degenerative disease, now equally prevalent; some due to real increase, but mostly a manifestation of demographic changes resulting in a higher proportion of old people. New hazards also have arisen as the pattern of life changes with "development"; carcinoma of the lung can be related to excessive smoking and smoke-laden atmospheres; suicide, alcoholism, and supposedly stress diseases are directly or indirectly nervous phenomena, the response of the human to the strains of a highly organized society. There is still much barring the way to Utopia.

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TOXICOLOGY OF HERBICIDES

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I. INTRODUCTION

In their endeavours to raise productivity, agriculturists have made use of chemicals to a steadily increasing extent. For the purpose of controlling injurious insects, fungi, weeds, *etc.*, a great number of pesticides has been developed, among which the insecticides, especially the organophosphate cholinesterase inhibitors, have attracted considerable toxicological interest. Relatively little has been written about herbicides, *i.e.*, compounds which have been found useful for weed control. Many of the toxicological data underlying assessments of the risk involved by using them in practice originate from confidential, non-published reports placed at the disposal of the authorities concerned. Such data have not been included in the present survey. Of a number of compounds, the toxicology of which has been elucidated in relation to their use for purposes other than as herbicides, only a brief account will be given with references to relevant literature.

Regarding the practical use of herbicides, reference is made to handbook literature (70, 80, 119, 120). Weedkillers usually are classified into two main groups, selective and non-selective. The selective substances can be used on crops without damaging the cultivated plants, whereas the non-selective types kill all vegetation. Substances of the latter group may therefore be used also for destroying potato haulm and for desiccation of green parts of plants which may unduly delay harvesting, in which case they are often called desiccants or defoliants. The herbicides are grouped, according to their mode of distribution in

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the weeds, into contact herbicides, which are active only at the site of application, translocated herbicides, which are distributed throughout the whole plant from exposed parts of foliage or roots, and residual herbicides, which are spread on or in the soil and are effective mainly against germinating seeds.

For the present survey of the toxicology of the herbicides, a classification according to chemical configuration has been chosen. Inasmuch as common names, abbreviations, and registered trade marks • are used indiscriminately in the literature, we have, as far as possible, included the common names recommended for pesticides by the British Standards Institution, marked (*), names approved by the British Weed Control Council (†), and those approved by the Weed Society of America (‡).

II. INORGANIC HERBICIDES

Prior to the Second World War, mainly inorganic compounds were used for chemical weed control. However, as their actions are not very selective and they are often very persistent in the soil, so that damage to cultivated crops has been difficult to avoid, they have been replaced by organic compounds to a steadily increasing extent. Yet, various familiar inorganic compounds are still used as herbicides, *e.g.*, calcium cyanamide, cupric sulphate, ferrous sulphate, mercurous chloride, potassium cyanate, and sodium tetraborate (borax). The toxicological data on these are well known from the handbook literature. The same is true of arsenites, sodium chlorate, and sulphuric acid, but these compounds will nevertheless by described briefly, because their use as herbicides has introduced special problems of toxicity.

A. Arsenites

Arsenites are used as non-selective herbicides, especially for destruction of potato haulm, as aqueous solutions of potassium-ortho-arsenite ($K_3As_3O_3$) and sodium ortho-arsenite ($Na_3As_3O_3$). The preparations may also contain meta-arsenite and pyro-arsenite alkali salts.

In rats, the LD50 for alkali arsenites has been found to be 70 mg/kg when administered by mouth and 150 mg/kg when applied dermally (32). In domestic animals (40) and man (105) the toxicity is considerably higher, fatalities having occurred in the horse, cow, and man after oral ingestion of 2 to 10 mg/kg. The solubility, preparation, and purity have a considerable influence on the toxicity of arsenic compounds (48); the toxic properties, actions on the animal organism, manifestations of poisoning, *etc.*, have been excellently summarized recently (108).

The use of arsenites as weedkillers has occasioned an extensive series of acute, often fatal cases of poisoning in domestic animals, especially cattle, which have eaten contaminated crops or residues of sprays (40). Concentrations toxic to man have been found in milk from cows fed on crops contaminated by arsenites (61). Cases of direct human poisoning have also been observed. An instance has been described where one woman died and four others fell ill after drinking water from a reservoir which had been contaminated through a leaking pump valve in the

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tank of an apparatus for spraying arsenite weedkiller. The drinking-water contained 180 p.p.m. (parts per million) of As (7, 8). This instance of poisoning in Great Britain resulted in a voluntary agreement between industry and the authorities for discontinuing the use of arsenite herbicides (32).

B. Sodium chlorate

Sodium chlorate and other chlorates are extensively used for killing all vegetation of farmyards, railway tracks, roadsides, *etc.*, as well as for destruction of potato haulm. Chlorates possess no particularly great acute toxicity, the LD50 for sodium chlorate administered by mouth to rats having been stated to be 1200 mg/kg (32). Nevertheless, oral ingestion of both sodium chlorate and potassium chlorate, used in oral hygiene, has caused numerous cases of poisoning in man (21, 33, 105). In domestic animals, too, cases of acute poisoning have been observed after oral ingestion of chlorates (40). The mechanism of the poisoning—methemoglobin production and its consequences—has been well studied (21, 33, 106).

A special problem is that of the high inflammability of sodium chlorate. This can be reduced in commercial preparations by admixture with calcium chloride or sodium chloride, but after dissolution in water and spraying, dried residues may become ignited. In Denmark, a tractor driver whose clothes had become impregnated with sodium chlorate during spraying, died of severe burns after they were ignited by a red-hot exhaust pipe.

C. Sulphuric acid

Sulphuric acid, which in certain countries, *e.g.*, Great Britain, is commonly used for potato haulm destruction, is often applied in high concentrations. Accidents with severe skin burns and eye burns may therefore occur (32). Inhalation of sulphuric acid may also exert a toxic effect, as indicated by studies on guinea pigs (3, 4) and on volunteer human subjects (5).

III. ORGANIC HERBICIDES

A. Chlorinated phenoxy-acids

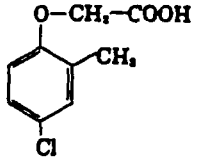
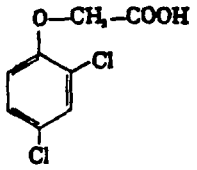
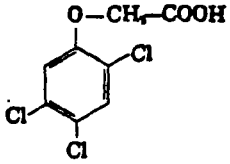
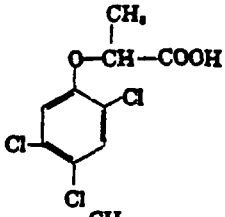
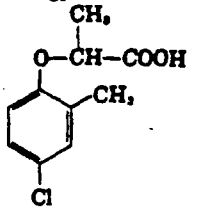
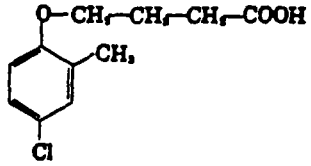
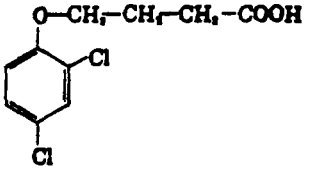
Chlorinated compounds of phenoxy-acetic acid, propionic acid, and butyric acid, have been very extensively used within the past 15 years as herbicides under the common name of "hormone weedkillers." They stimulate parts of susceptible plants, particularly within the group of dicotyledons, to excessive, uncontrolled growth, which causes the plants to die (109). The most important compounds, which in the forms of water-soluble salts or lipoid-soluble esters constitute the active components of commercial preparations, are listed in Table 1, where the LD50 values in rats are also given. In addition to the compounds listed in the table, *p*-chlorophenoxyacetic acid (4-CPA), sodium 2-(2,4-dichlorophenoxy)ethyl sulphate (2,4-DES-sodium, Sesone, SES), and γ-(2,4,5-trichlorophenoxy)butyric acid (2,4,5-TB, 4-(2,4,5-TB)) are used as herbicides of this type.

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TABLE 1

Chlorinated phenoxy-acids

Compound	Formula	Common or Abbreviated Names	LD50, mg/kg by Peroral Administration to Rats	Ref. No.
4-Chloro-2-methyl-phenoxy-acetic acid		*MCPA*,*†‡ MCP, 4K-2M	700	(89)
2,4-Dichloro-phenoxy-acetic acid		*2,4-D*†‡	375	(89)
2,4,5-Trichloro-phenoxyacetic acid		*2,4,5-T*†‡	500	(89)
α-(2,4,5-Trichloro-phenoxy)propionic acid		*2,4,5-TP*,† Silvex‡	650	(89)
α-(4-Chloro-2-methyl-phenoxy)propionic acid		*Mecoprop*,*† 2-(MCPP),‡ CMPP, MCPP	700–1500	(32)
γ-(4-Chloro-2-methyl-phenoxy)butyric acid		*MCPB*,*† 4-(MCPB)‡	700–1500	(32)
γ-(2,4-Dichloro-phenoxy)butyric acid		*2,4-DB*,*† 4-(2,4-DB)‡	700–1500	(32)

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The acute toxicity following oral administration to a number of experimental animals is moderate. The LD50 as a rule is of the order of 300 to 700 mg per kilogram for the species examined (89), excepting dogs, which seem to be more susceptible, where the LD50 for both (2,4-dichlorophenoxy)acetic acid (2,4-D) and 2,4,5-T has been found to be 100 mg/kg (27). Experiments with oral administration of various salts and esters of 2,4-D as pure chemicals and as commercial preparations showed no significant difference in toxicity to a number of small animals from that of the free 2,4-D acid (53, 89).

Short-term studies on rats revealed no signs of reduced intake of food or inhibition of growth in response to admixture of 2,4-D at 400 p.p.m. in the fodder for 30 days or 1000 p.p.m. for 14 days. Subcutaneous injection of 50 to 100 mg 2,4-D per kg to mice daily for 90 days had no demonstrable effect on the general condition, fertility, or tissue histology (53). In Table 2 are recorded the results of feeding phenoxy-compounds to larger animals. Except for the rather considerable toxic effect on sheep of 2,4,5-TP, administered in the form of propylene glycol butyl ether esters, these experiments showed that the large herbivores seem to tolerate prolonged ingestion of phenoxy-compounds to the same extent as rats. In dogs, on the other hand, these compounds were found to have a pronounced toxic action: 20 mg/kg given for 2 to 3 weeks produced severe, fatal poisoning.

Parenteral and oral administration of toxic doses of 2,4-D to experimental animals brings about a characteristic complex of signs and symptoms (18, 27, 53), which has been studied particularly in dogs. After a few hours the animals display a disinclination to move. This passiveness is gradually aggravated, and a picture of myotonia develops with rigidity of the skeletal muscles and ataxia. The condition may improve transitorily with movement. In severe cases the animals show progressive apathy, depression, and muscular weakness, especially of the hindlegs, with periodic, clonic spasms, and, finally, coma. The muscular signs are accompanied by marked anorexia; frequently, irritation of the nose and eyes is indicated by scratching reactions. Further, bleeding from the nose and mouth may occur, as well as diarrhea with blood-stained stools. Local irritation of the alimentary tract often causes vomiting. However, this sign may be absent, even after oral administration to dogs (27). Autopsy may reveal necrotic ulcers of the oral mucosa and signs of irritation with histologically demonstrable inflammatory changes and necrosis of the small intestinal mucosa, as well as focal necrosis in the liver (27, 53) and degeneration of the renal tubules.

When given by mouth to dogs, even in fatal cases 2,4,5-T has produced only weak signs in the forms of ataxia and stiff movements of the hindlegs (27).

As for the human response, a report is available (9, 75) of a man who in a self-experiment consumed 500 mg of 2,4-D daily for 3 weeks, with no perceptible effect. A case of acute fatal poisoning has been reported from Denmark, where a young farm worker committed suicide by oral ingestion of not less than 6500 mg of 2,4-D. When found, the body showed signs of having been subject to violent convulsions before the occurrence of death. The changes post mortem were unspecific hyperemia of the lungs, liver, and brain (78).

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TABLE 2

Chlorinated phenoxy-acids; short-term studies in dog, sheep, and cow

Compound	Animal Species	Daily Dose, mg/kg Body Weight	Result	Ref. No.
MCPA	Cow	30	Tolerated without detectable symptoms during 21 days	(24)
2,4-D	Sheep	100	Tolerated without detectable symptoms during 35 days	(85)
—	Dog	2–10	Tolerated without detectable symptoms during 90 days	(27)
—	Dog	20	3 of 4 animals died, 18th to 49th day	(27)
2,4,5-T	Sheep	100	Tolerated without detectable symptoms during 35 days	(85)
—	Dog	2–10	Tolerated without detectable symptoms during 90 days	(27)
—	Dog	20	4 of 4 animals died 11th to 75th day	(27)
2,4,5-TP	Sheep	100	Lethal after 11 doses	(55)

The action of ingested phenoxy-acid herbicides on muscular function, which is reminiscent of that following administration of halogenated acetic acid compounds (22, 23), suggests an interference with carbohydrate metabolism. Two cases of a transitory diabetiform condition observed in spraying personnel following work with chlorinated phenoxy-acid herbicides point in the same direction. However, hyperglycemia and glycosuria could not be reproduced with certainty in rabbits; only one out of five animals responded in this way in exploratory experiments, where the doses ranged from 125 to 500 mg/kg and the period of administration from 6 to 50 days (68).

The results of a long series of investigations into the toxicity to domestic animals and game of herbicidal preparations and treated crops suggested that acute poisoning caused by consumption of crops from sprayed fields is unlikely to occur (16, 24, 46, 75, 87), excepting that "grass-eating" dogs may be poisoned by newly sprayed lawns. Human beings and domestic animals are presumably liable to poisoning only by oral ingestion of highly concentrated preparations or spray solutions.

A special problem in connection with the use of chlorinated phenoxy-acid compounds is the tendency of the preparations—even in extremely low concentrations—to impart to water, milk, and other nutrients a very persistent chlorophenol-like odor and taste. Spilling of highly concentrated preparations close to wells or water courses has in several instances, by percolation through the strata, given a disagreeable taste to the water for long periods afterwards. Administration of 2,4-D to dairy cattle does not seem to result in excretion of biologically demonstrable amounts in the milk (75).

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Very few toxicological data are available regarding other chlorinated *aromatic* acids, introduced alone or in combination as pre-emergence and selective herbicides for agricultural crops. 2,3,6-Trichlorophenyl-acetic acid (fenac‡), 2,3,6-trichlorobenzoic acid (2,3,6-TBA††, TBA, TCB) and 3-amino-2,5-dichlorobenzoic acid (*amiben*‡) are used for these purposes. The LD50 of 2,3,6-TBA after a single oral dose to rats was stated to be within the range of 700 to 1500 mg/kg (32).

B. Chlorinated aliphatic acids and their sodium salts

The chlorinated *aliphatic* acids possess more general phytotoxic properties. Some of these are used, therefore, for defoliation and desiccation of cultivated plants prior to mechanical harvesting. The most important compounds are: sodium trichloro-acetate (TCA-†‡ sodium), sodium monochloro-acetate (SMCA), and sodium-dichloropropionate (Dalapon-sodium*†‡), as well as the combined compound, 2-(2,4,5-trichlorophenoxy)-ethyl-,2′,2′-dichloropropionate (Erbon†‡).

Studies on the acute toxicity of *sodium trichloro-acetate* have shown that this substance is not very toxic (118), the LD50 after oral administration being 3320 mg/kg for rats and 4970 mg/kg for mice. The animals quickly go into an anesthesia-like state, which lasts for 36 hours, to be succeeded by coma and death, or by awakening and survival. Sodium trichloro-acetate has a far less local-irritating action than the free acid, which can corrode the skin and mucous membranes.

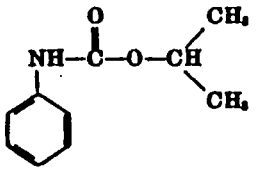
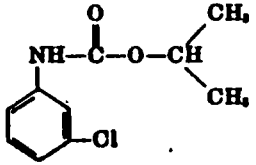
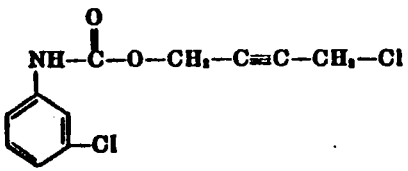
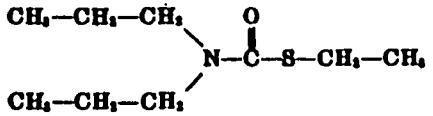
Sodium monochloro-acetate (SMCA) is considerably more toxic. Its action on bacteria and in the animal organism is reminiscent of that of other monohalogen-substituted acetic acid compounds, such as monoiodo- and monobromacetic acid, the enzyme-inhibiting and bacteriostatic properties of which have been thoroughly studied (*vide* 22). The LD50 after oral administration has been found to be 76 mg/kg for rats, and 80 mg/kg for guinea pigs (118), and of the same order for geese (20), whereas for mice the toxicity is somewhat lower, the LD50 being 255 mg/kg (118). In the small experimental animals apathy and loss of weight were noticed, and in the fatal cases death occurred within 3 days. The fatal oral dose for young cattle was found to lie within the range of 100 to 150 mg/kg. Colicky-like restlessness and incoordination were observed, developing in the course of 4 to 5 hours into universal fascicular twitchings, gnashing of the teeth, anxiety, dyspnea, and tachycardia. Coma and death followed after 9 hours (23). This picture, which is probably referable to blocking of oxidative metabolic processes, is reminiscent of that seen in pigs (22) and dogs (6) poisoned with monobromacetic acid. The gross and microscopic findings at autopsy were likewise identical with those described for monobromacetic acid-poisoned pigs (22).

Sodium 2,2-dichloropropionate (*Dalapon*) has become of particular interest owing to its herbicidal action on monocotyledons, such as grasses, in relatively small quantities, therefore being useful as a selective herbicide in certain crops of cultivated plants. Dalapon is absorbed by plants and then translocated. On this

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TABLE 3
Carbamates

Compound	Formula	Common or Abbreviated Names	LD50, mg/kg by Peroral Administration to Rats	Ref. No.
Isopropylcarbanilate, isopropyl-N-phenyl-carbamate		Propham,*† IPC‡	1000	(119)
Isopropyl-m-chloro-carbanilate, isopropyl-N-(3-chloro-phenyl) carbamate		Chloropropham,*† CIPC,‡ chloro-IPC	5000	(115)
4-Chlorobut-2-ynyl-m-chlorocarbanilate, 4-chloro-2-butynyl-N-(3-chlorophenyl) carbamate		Barban(e),‡ Carbyne●	600	(36)
Ethyl-NN-dipropylthiolcarbamate		EPTC,‡ Eptam●	3160	(100)

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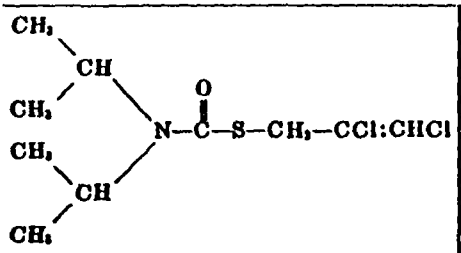
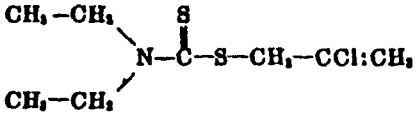
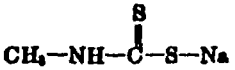
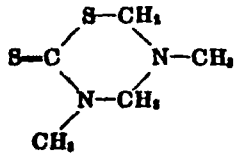
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2,3-Dichloroallyl-di-isopropyl thiolcarbamate
2-Chloroallyl-NN-diethyl-dithiocarbamate
Sodium N-methyl dithio-carbamate dihydrate
3,5-Dimethyltetrahydro-1,3,5,2H-thiadiazine-2-thione

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	Avadex®	395	(119)
	*CDEC*,†‡ Vegadex®	850	(119)
	SMDC,‡ metham-sodium, Vapam®	820	(29)
	DMTT,‡ Mylone®	500	(119)

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account, prolonged feeding experiments have been conducted with a view to assessing the possible risk involved by ingestion of residues in vegetables. The toxicity of the substance has been studied from several aspects through a comprehensive series of experiments (81). Administration of single doses to a number of small experimental animals has given LD50 values for rats, mice, guinea pigs, rabbits, and chickens within the range of 4000 to 9000 mg/kg. Two young cattle survived 1000 mg/kg given by mouth daily for 10 days. Beyond transitory symptoms in one of these (anorexia, diarrhea, indisposition), no signs of a toxic action were found by clinical or pathological examination. After oral administration of 15, 50, and 100 mg/kg daily to dogs for one year, blood and urine analyses, as well as liver function tests and histological examination of tissues revealed no signs of a toxic action, beyond an increased weight of the kidneys following the dose of 100 mg/kg. Examination of tissue specimens from the dogs showed up to 78 p.p.m. of Dalapon in kidney and liver tissues (81).

Feeding of rats with Dalapon for 2 years in doses of 100, 300, and 1000 p.p.m. in the fodder, corresponding to about 5, 15, and 50 mg/kg daily, was tolerated with no signs of a toxic action, apart from a minor increase in kidney weight on the largest dose. In these experiments with 1000 p.p.m., 10 to 30 p.p.m. of Dalapon was found on chemical analysis of the liver and kidney, and 20 p.p.m. in milk. Thus, cumulation does not take place. In rats, reproduction and lactation proved to be uninfluenced through three generations with daily administration of 300 to 3000 p.p.m. of Dalapon in the fodder (81).

The local-irritating action of Dalapon has been studied on rabbits, the skin of which was exposed daily for 10 days to a 10% aqueous solution. No more than signs of a mild, transitory irritation was seen (81).

C. Carbamates and allyl alcohol

Within this group of herbicides, which, as shown in Table 3, comprises carbamates, thiocarbamates, and dithiocarbamates, is a number of compounds which are used especially as pre-emergence or selective herbicides on certain crops. Propham and chlorpropham have also been used to prevent potatoes intended for consumption from sprouting while stored. The toxicity of these compounds with prolonged ingestion is therefore of particular interest.

Propham (O-*Iso*propyl N-phenyl carbamate, IPC). Various investigations have shown that carbamate esters possess a carcinogenic action. Thus intraperitoneal injection of *iso*propyl carbamate raised the frequency of lung tumors in a strain of mice from 17 to 90% (66). The idea of inquiring into a possible carcinogenic action of Propham, which splits off aniline by acid hydrolysis, therefore suggested itself. However, with prolonged oral, intramuscular, and intrapleural administration of Propham to rats and mice, no signs of a carcinogenic action were found (56). This is in agreement with the results of experiments on groups of four rats each, given 400, 800, and 1600 p.p.m., respectively, daily in the fodder for 3 months. No signs were demonstrable here of a toxic action on the general condition, growth, or fertility, and no pathological changes were seen at autopsy or on histological examination of the tissues (101).

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Experiments with the chemically closely related *Chlorpropham* (*iso*propyl-N-[3-chlorophenyl] carbamate, CIPC) have shown it to have a very low acute toxicity to rats and rabbits, LD50 by oral administration having been found to be of the order of 5000 mg per kg for both species (115). In feeding experiments on male rats given 310 to 20,000 p.p.m. for 90 days, no effect was observed on growth. Doses of 1250 p.p.m. and higher produced an increase in weight of their livers, without histological changes being demonstrable (116).

In the light of the above investigations, the observation of the development of skin tumors following painting of the back skin of Propham- and Chlorpropham-fed rats with croton oil is of considerable theoretical interest (34). However, comprehensive feeding experiments (67) with administration of 2000 p.p.m. through 2 years to rats and 1 year to dogs revealed no carcinogenic effect. Feeding at 20,000 p.p.m. provoked in both species signs of a toxic action, which was manifested in rats by retarded growth, increased mortality of the male animals, and increased liver and kidney weights, though without demonstrable histological changes. In dogs, retarded growth, increased weights of liver, kidney, and spleen, and splenic congestion were noted (67).

Barbane (4-chloro-2-butynyl-N-[3-chlorophenyl]carbamate) displays a somewhat greater acute toxicity than the Propham compounds, the LD50 after oral administration having been found to be 600 mg per kg for the rat and rabbit, and 240 mg/kg for the guinea pig. Dermal application of 1600 mg/kg over a period of 24 hours caused no deaths among rats. Oral administration of 9, 19.5, and 37 mg/kg daily for 22 days produced no toxic reaction, whereas 75 mg/kg over the same period effected loss of weight. Feeding experiments with rats showed no toxic action of 150 p.p.m. for 18 months (36). Barbane is a potent skin-sensitizing agent in man, in whom allergic reaction with rash develops at subsequent contact. Protection against cutaneous contact is therefore necessary during its use. Plastic (polyvinyl chloride) seems to be a more suitable protective material than rubber (36).

SMDC (sodium N-methyl dithiocarbamate dihydrate, metham-sodium) has a special sphere of application, being used for killing weed seeds, soil nematodes, and the like by so-called soil sterilization. When applied in the soil it liberates gaseous *methylisothiocyanate*, the active substance, which is also available commercially as an aqueous solution (Trapex) for similar purposes. The LD50 of SMDC by oral administration to rats has been stated to be 820 mg/kg (29). The toxicity of *methylisothiocyanate* is considerably higher, the LD50 being 97 mg/kg (82). The main toxicological interest attaches, however, to the pronounced locally irritating action of these substances on the skin and mucous membranes, especially those of the respiratory organs, as well as the possibility of absorption of toxic amounts by these routes. The LD50 of SMDC after dermal application to rabbits has been found to be 800 mg/kg (29), whereas daily rubbing of methylisothiocyanate in 10% ethanol for 9 weeks into rabbit ears gave no more than a weak reaction (82).

Allyl alcohol. This unsaturated alcohol has the same range of application as SMDC and methylisothiocyanate; it is applied in solution to the soil, where its

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action is strong, but of short duration, so that cultivated plants can be sown about two weeks later and grow without the interference of weed seedlings. Allyl alcohol has a strong locally irritating action and can be absorbed through the alimentary tract, the lungs, and intact skin. Extensive investigations of its toxicity have been made on experimental animals. The early literature, to which Miessner (74) contributed significantly by his investigations from 1891, has been summarized thoroughly (79). The toxicity and mechanism of poisoning have been studied in the United States within recent years (30, 65, 94, 95). LD50 values after oral administration to the mouse, rat, and rabbit ranged from 50 to 100 mg/kg, and after percutaneous administration to rabbits, from 45 to 90 mg/kg. Some time after the administration the animals were found apathetic, but not anesthetized, as they reacted strongly to pain stimuli. Lacrimation was seen, and often also increased secretion by salivary and gastroenteric glands, increased motility of the intestines, dyspnea due to pulmonary effusion, loss of weight, hemoconcentration, and vasodilatation. Autopsy revealed congestion of the organs as well as periportal necrosis (30, 65). Local application to rabbit skin produced only transitory signs of irritation. Instillation into the eyes of rabbits resulted after 1 hour in redness and swelling, which were intensified in the course of 24 hours. In some instances keratitis was seen, but the condition returned to normal within a week (30).

In acute inhalation experiments on rats, half of the exposed animals died in the course of 1 hour at an air concentration of 1060 p.p.m.; 165 p.p.m. for 4 hours or 76 p.p.m. for 8 hours gave the same result. Experimental volunteers exposed to air concentrations of 0.78 to 25 p.p.m. for 5 minutes one to three times weekly over a period of 50 days, developed no signs of pulmonary discomfort or affection of the CNS. The subjects could barely detect 0.78 p.p.m., while irritation of the nasal mucosa was first noticed at 6.25 to 12.5 p.p.m., and eye irritation at 25 p.p.m. (30).

Acrolein (acrylic aldehyde), the aldehyde corresponding to allyl alcohol, has been used for control of water plants and algae by introduction under water. The substance has the disadvantage of being highly toxic to fish, but this seems to be compensated for by its quick elimination. The LD50 after single subcutaneous administration is 30 mg/kg for mice and 50 mg/kg for rats. Moderate anesthesia occurs, as well as a few convulsive fits and dyspnea, especially in mice. Autopsy with histological examination of tissues has revealed pulmonary edema, chiefly perivascularly and hyperemia, slight fatty degeneration of the liver, and focal inflammatory processes in the kidney. At poisoning after inhalation, where 0.3 p.p.m. for 30 minutes has a lethal effect, the histological changes—edema, hyperemia, and epithelial cell degeneration—are confined to the lungs (95).

D. Substituted ureas

The most important compounds belonging to this group of herbicides have been listed in Table 4, in which it is shown also that these compounds have a low acute toxicity when administered by mouth to rats. For 3-(*p*-chlorophenyl)-1,1-

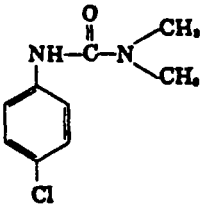
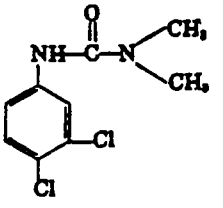
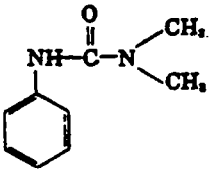
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TABLE 4

Substituted ureas

Compound	Formula	Common or Abbreviated Names	LD50, mg/kg by Peroral Administration to Rats	Ref. No.
N-(p-Chlorophenyl)-N¹N¹-dimethylurea		Monuron,*†‡ CMU	3500	(28)
N-(3,4-Dichlorophenyl)-N¹N¹-dimethylurea		Diuron,†‡ 3,4-DDU, DMU	3400	(28)
NN-Dimethyl-N¹-phenylurea		Fenuron,†‡ PDU	7500	(91)

dimethylurea (Monuron) the approximate lethal dose by single oral administration to guinea pigs and rats has been set at 670 and 1500 mg/kg, respectively (28). To the same group belong also N-butyl-N¹(3,4-dichlorophenyl)-N-methylurea (Neburon†‡), 1,3-di(2,2,2-trichloro-1-hydroxyethyl)urea (Dichloral urea, DCU‡, DU), and N-cyclooctyl-N¹N¹-dimethylurea (OMU).

The substituted urea derivatives destroy numerous species of plants, and their activity persists for a long time. They are used in large doses for eradication of all vegetation of farmyards, railway tracks, roadsides, *etc.* They may also be used in much lower concentrations on cultivated crops, such as asparagus. Owing to the long persistence of these substances, residues may be present in harvested crops. In feeding experiments on rats given Monuron at 25, 250, and 2500 p.p.m. for 2 years, no effect was observed on survival or frequency of tumors, as compared with control groups and in the colony of normal rats. The conclusion was drawn that "there was no indication that Monuron is cancerogenic" (54). In the group given 2500 p.p.m. the male rats were seen to lose weight after only one month of feeding. During the experiment mild anemia was also found, and at the end the

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weights of the liver and spleen had increased. On histological examination of tissue specimens none of the groups presented changes attributable to an action of Monuron. In experiments on dogs, which received in their diet 2.5, 12.5, and 25 mg Monuron per kg for one year, no signs of toxicity were seen during the experiment, and at autopsy with histological examination of tissue specimens no changes were noticed that seemed to originate from feeding with Monuron (54).

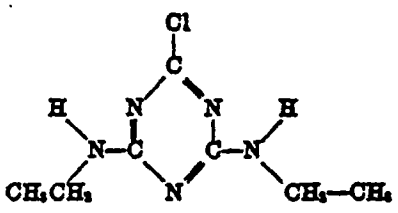
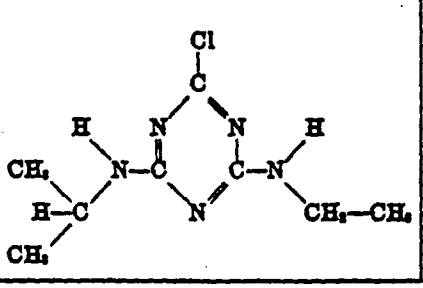
Rats given 500 p.p.m. of *Fenuron* in the diet for 90 days tolerated this dose with no signs of poisoning (91). *Diuron* was likewise tolerated at 50 p.p.m. While 5000 p.p.m. of this compound for 90 days caused no deaths, but loss of weight, a fall in the number of red blood corpuscles, and pathological changes in the spleen were noted (28).

Preliminary experiments using Monuron and Diuron on guinea pigs have shown no signs of allergic skin sensitization. Neither have such reactions been observed in experimental subjects or factory employees working with these compounds (28).

E. Triazines

The announcement of the phytotoxic and plant-growth-regulating properties of a series of aminotriazines (41), especially the 2-chloro-2,4-bis(ethylamino)-1,3,5-triazine compound, Simazine, initiated the synthesis of a great number of herbicidal derivatives. The two most commonly employed of these are shown in Table 5, whereas the compounds known under the names of: Propazine‡(2-chloro-

TABLE 5
Triazines

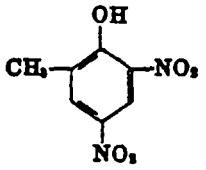
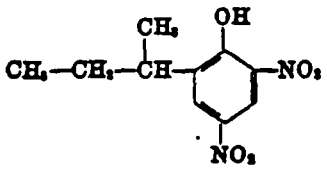
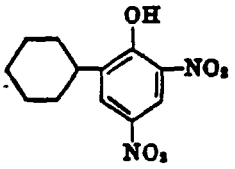
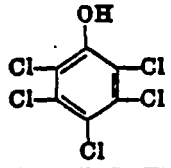
Compound	Formula	Common Names	LD50, mg/kg by Peroral Administration to Rats	Ref. No.
2-Chloro-4,6-bis (ethylamino)-1,3,5-triazine		*Simazine*†‡	5000	(42)
2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine		*Atrazine*‡	1750	(43)

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TABLE 6
Substituted phenols

Compound	Formula	Common or Abbreviated Names	LD50, mg/kg by Peroral Administration to Rats	Ref. No.
2-Methyl-4,6-dinitrophenol		*DNOC*,*† DNC,‡ dinitrocresol	40	(32)
2-s-Butyl-4,6-dinitrophenol		*Dinoseb*,*† DNBP,‡ DNOSBP	50	(32)
2-Cyclo-hexyl-4,6-dinitrophenol		*Dinex*, DNOCHP		
Pentachlorophenol		PCP†‡	80–200	(32)

4,6-*bis*[*iso*propylamino]-1,3,5-triazine), Trietazine‡ (2-chloro-4-diethylamino-6-ethylamino-1,3,5-triazine), Ipazine‡ (Isodiazine, 2-chloro-4-diethylamino-6-*iso*propylamino-1,3,5-triazine), Simetone‡ (2,4-*bis*[ethylamino]-6-methoxy-1,3,5 - triazine), Prometone‡ (2,4 - *bis*[*iso*propylamino] - 6 - methoxy - 1,3,5-triazine), and Atratone‡ (2-ethylamino-4-*iso*propylamino-6-methoxy-1,3,5-triazine), are still being used mainly experimentally.

These compounds are almost insoluble and persist for a long time in the soil. They have been used mainly for eradication of all vegetation as well as for local weedkilling under fruit trees and shrubs. Certain species of plants, *e.g.*, asparagus and maize or corn, are, however, resistant to the herbicidal action of the triazines. Their use on areas with these cultivated plants is therefore practicable but involves a risk of residues in crops for consumption.

Studies on the toxicity of *Simazine* (42) showed that the LD50 after oral administration to mice, rats, rabbits, chickens, and pigeons in all cases exceeded 5000 mg/kg, whereas all rats survived daily doses of 2500 mg pure Simazine per kg for 4 weeks, and 1250 mg of a commercial preparation per kg. Rats fed for two years on a diet containing 1, 10, and 100 p.p.m. of Simazine revealed no signs of

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toxicity (46a), and screening tests for various acute actions, such as spasmolysis on the isolated intestine and analgesia in mice, did not demonstrate significant activity (46a).

The LD50 of *Atrazine* (2-chloro-4-ethylamino-6-*iso*propylamino-1,3,5-triazine) (43) after a single oral administration was found to be 1750 mg/kg for mice and 3080 mg/kg for rats, while a daily dose of 400 mg/kg through 6 weeks proved fatal to half of the test rats.

F. Substituted phenols

Dinitrophenols. The checkered history of the dinitrophenols also includes a chapter on their applicability as herbicides. In agriculture these compounds are used also as insecticides, acaricides, and ovicides, for potato haulm destruction, pre-harvest desiccation of leguminous seed crops and apple-thinning sprays. The chances of contact are therefore great, and their users are often poisoned when protective measures are disregarded.

Three derivatives are commonly used: DNOC (DNC, 2-methyl-4,6-dinitrophenol), Dinoseb (DNPB, 2-sec.butyl-4,6-dinitrophenol), and Dinex (DNOCHP, 2-cyclohexyl-4,6-dinitrophenol). The chemical configurations and LD50's for rats after oral administration are shown in Table 6. The mechanism of toxic action which is based on the uncoupling of oxidative phosphorylation, has been studied in detail; a survey has been given in this journal (17). A great number of toxicological data have been given in Handbook of Toxicology (77), and poisoning of man and domestic animals has been reported in recent surveys (12, 32, 40, 72, 86, 88). Clinical accounts are available from Belgium (52) and Great Britain (15), among others. Up to 1950 seven British cases had been reported of fatal poisoning with DNOC used as a weedkiller. Countermeasures against poisoning (14) have since been satisfactory in Britain, no fatal accidents having occurred after their introduction (12, 32).

Pentachlorphenol. Like the dinitrophenols, pentachlorphenol is an effective uncoupler of oxidative phosphorylation. The mechanism of toxic action has been studied in experiments with molluscan and mammalian tissues as well as on enzymes (110, 111, 112, 113). It seems to be due to inhibition of the intracellular transfer of energy-rich phosphates as well as their synthesis. In agreement with this, the symptoms of intoxication include acceleration of respiration, hyperpyrexia, hyperglycemia, glycosuria, and promptly occurring motor weakness. As in cases of dinitrophenol poisoning, *rigor mortis* sets in early and is very pronounced (26, 45, 64, 71).

The acute toxicity of pentachlorphenol as well as that of its sodium salt have been studied by oral, subcutaneous, and dermal administration of single doses to rats and rabbits (26, 64, 71, 102), and by single subcutaneous injections into dogs (71). The LD50's and the minimum lethal doses were found to vary considerably, ranging from 27 to 550 mg/kg. The highest toxicity was noted for pentachlorphenol dissolved in mineral oil (fuel oil) given by mouth to rats, and the lowest for the sodium salt administered in the same way to rabbits. After dermal application, mineral oil solutions are likewise more toxic than solutions in vegetable

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oil and in water (64). Furthermore, the local reaction, which may manifest itself by changes from erythema to grave dermatitis, is stronger with mineral oil as vehicle (64, 71).

The results of short-term studies on a number of animal species suggest a certain degree of cumulative action, especially after subcutaneous injection (71); signs of such have also been seen after oral administration to rats and cats (26). In experiments on calves, on the other hand, cumulative action has not been observed (51).

From the practical use of pentachlorphenol, this compound is well known as a skin irritant (12); in addition, toxic amounts may be absorbed through the skin. In relation to its use as a timber-preservative, human cases of poisoning have occurred, some of which have been fatal (73, 107).

Sodium pentachlorphenate in aqueous solution with mineral oil added, used as a herbicide in pineapple plantations in Australia, has been described as the cause of poisoning of nine workers, five of whom died. In one, who died after 21 hours, pentachlorphenol was demonstrated by chemical analysis of tissue specimens in quantities of 2 to 14.5 mg per 100 g tissue (45).

In cattle, cases of poisoning have been described which ran a fatal course within 24 hours of oral ingestion of 5 % pentachlorphenol in kerosene (97). Mild signs and symptoms of poisoning in sheep and cattle have followed 25 mg/kg (85).

G. Miscellaneous organic herbicides

1. Tributyl phosphorotrithioate (DEF). Organophosphates have become very extensively used as insecticides within agriculture and horticulture. A comprehensive literature is available on the toxicity of these compounds, related to their cholinesterase-inhibiting action (55). In studying DEF, which is used as a defoliant in cotton fields, attention has been focused on the relationship between the toxicity and the inhibiting action on the cholinesterase activity in different organ tissues (76). The LD50 after oral administration to rats is 325 mg/kg (Table 7). With daily intraperitoneal injections of 50 mg/kg into rats for up to 60 days no signs of poisoning were observed, although after sacrifice cholinesterase of brain tissue was found to be 14 % of the average in control groups. Injection of 100 mg/kg was lethal after 5 to 30 days. In acute poisoning experiments depression and inactivity were seen after one hour. These symptoms persisted for 24 to 48 hours, unless irritability or manifestation of pain or tremor was produced by external stimuli. The depression developed in some cases into muscular weakness with rigidity. Not till 2 to 6 hours after injection did the animals also display the usual signs of anticholinesterase poisoning: profuse urination, salivation, and lacrimation, and respiratory impairment increasing to respiratory paralysis and death (76). The cholinesterase activity of the red blood cells and brain was often poorly related to the cholinergic signs, often with freedom from any signs or symptoms despite a low activity level (76). Having been observed also in cases of poisoning with other organic phosphorus compounds (39, 63), this fact does not militate against the hypothesis of cholinesterase inhibition as an etiological factor in the mechanism of poisoning. The somewhat differing manifestations of poisoning, and the failure of prophylactic and therapeutic effects of enormous

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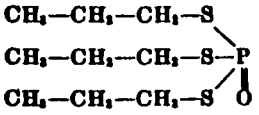
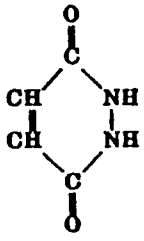
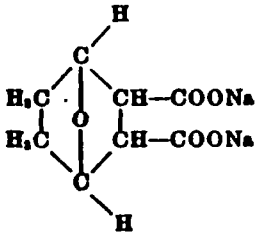
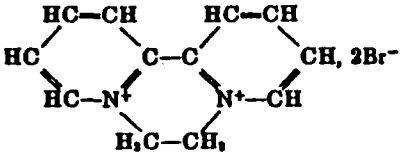
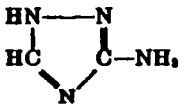
Compound
S,S,S-tributyl phosphorotrithioate
1,2,3,6-tetrahydro-3,6-dioxopyridazine
Disodium 7-oxabicyclo-(2,2,1)heptane-2,3-dicarboxylate, Disodium 3,6-endoxohexahydrophthalate
9,10-dihydro-8a,10a-diazoniaphenanthrene dibromide, 1,1'-ethylene-2,2'-dipyridylium dibromide
3-amino-1,2,4-triazole

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TABLE 7

Miscellaneous organic herbicides

Formula	Common or Abbreviated Names	LD50, mg/kg by Perotal Administration to Rats	Ref. No.
	*DEF*	325	(76)
	*Maleic hydrazide*, MH‡	4000	(13)
	*Endothal-sodium*††‡	80	(32)
	*Diquat*,•††‡ Reglone®	400–440	(83)
	Amitrole,‡ ATA, amizol, amitol	25000	(90)

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doses of atropine (76) suggest, however, that other factors also contribute towards the toxic action of DEF.

2. Maleic hydrazide (1,2,3,6-tetrahydro-3,6-dioxopyridazine) is used for spraying on roadsides, hedges, and the like, to facilitate and reduce the work with cutting, *etc.* The growth-inhibiting properties are utilized further by spraying the compound on root crops 2 to 4 weeks before harvesting, as this greatly reduces the tendency to sprouting during storage.

In experiments on a number of small animals, the acute and chronic toxicities of this compound were found to be low. Thus, for instance, the LD50 after oral administration to rats was 4000 mg/kg, while prolonged feeding experiments on rats with 10,000 p.p.m. of maleic hydrazide in the fodder showed no signs of a toxic action within the normal life span (13). The demonstration of the fact that in *Vicii faba* roots maleic hydrazide not only inhibited the growth of cells, but also caused breakage of the heterochromatin in the chromosomes (25) aroused a great interest in ascertaining whether animal cells might also be susceptible to this action, or whether it is specifically directed against plant cells. This is important, because the ability of substances to produce chromosome breakage may be accompanied by carcinogenesis. In thorough investigations, comprising weekly subcutaneous injections into mice and rats of 500 mg/kg for 100 weeks, as well as feeding experiments over the same period with 10,000 p.p.m. of maleic hydrazide in the diet, no signs of a carcinogenic action were demonstrable. Tests for a possible co-carcinogenic action on application together with croton oil to mouse skin revealed no greater tumorigenic action than that of the oil alone. Implantation of maleic hydrazide pellets in the bladder of mice did not give rise to tumor formation, and administration of 125 mg of the compound per kg to tumour-affected rats had no effect on the tumour growth or cell mitoses. In experiments with tissue cultures of mouse epidermis and guinea-pig skin, no effect was seen on cell division, mitosis, or tissue respiration (13).

The fate of maleic hydrazide has been studied in rabbits. After oral administration of 100 mg/kg, the rabbits excreted 43 to 62% unchanged in the urine in 48 hours. Coupling products were not detected, and the fate of the remaining 40% of the administered amount could not be determined (13).

3. Endothal-sodium (disodium 3,6-endoxohexahydrophthalate), which has strong plant-damaging properties, is used particularly as a defoliant and desiccant in leguminous crops, and has also been tried as a pre-emergence herbicide, *e.g.*, for sugar beets. The compound, which is closely related to cantharidin, is very toxic. After oral administration to rats, the LD50 has been found to be 80 mg/kg (32), whereas the lethal dose by intravenous injection into rabbits and dogs is of the order of 5 to 10 mg/kg (44). Shortly after administration, scratching of the nose was observed, and in the dogs, intermittent retching and vomiting also occurred for 2 hours, followed by death or recovery. In the rabbits, headshaking and irregular, greatly accelerated respiration were noticed (98). For both animal species, death was attributed to respiratory failure. In experiments with isolated ventricles of frog hearts and rabbit atria functional depression was seen in response to a 1 in 20,000 solution of Endothal-sodium (44). Hence, cardiac de-

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pression has been supposed to be the cause of death. However, intravenous injection of Endothal into anesthetized dogs, atropinized as well as non-atropinized, caused a fall in blood pressure and respiratory failure, whereas changes of the ECG were first noticed in association with severe respiratory impairment in the terminal phase. While cats reacted in the same way as dogs, although with a less pronounced fall in blood pressure, domestic fowls were found to be insensitive to doses four times larger than those which were active on dogs and cats (98). The effects of Endothal both *in vitro* and *in vivo* were found to be characterized by manifesting themselves only after a long latency (98). This points towards a metabolite as the proper active factor.

Application of a 10 to 20% aqueous solution to the skin produced erosions which developed into necrosis, in some cases resulting in death owing to cutaneous absorption (44). The LD50 after dermal application to rats was 750 mg/kg (32).

4. Diquat (*Reglone*). Diquat (9,10-dihydro-8α,10α-diazoniaphenanthrene dibromide) has been proposed as an agent to produce desiccation and defoliation prior to harvesting, destruction of potato haulm, and as a pre-emergence and a total herbicide.

The LD50 after oral administration to rats was 400 to 440 mg/kg, whereas the LD50 after subcutaneous injection was 20 mg/kg, with death occurring in the course of 5 to 7 days. At autopsy, the intestinal canal, especially the cecum, was distended; histological examination revealed signs of gastrointestinal irritation, and in the lungs, thickened alveolar walls, particularly after large doses (83).

Intraperitoneal injection of 500 mg/kg to rats produced cyanosis and convulsions, culminating in death after 2 hours. Subcutaneous injection of 1 mg/kg into rats for 3 weeks caused no signs of toxicity. Similarly, feeding experiments to rats over 14 months with 500 p.p.m. in the diet disclosed no carcinogenic or toxic action (83). Following oral administration of C^{14}-labelled Diquat, 90 to 97% of the amount of activity given was recovered in the feces in the course of 48 hours (83). This, correlated with the difference in toxicity after oral and parenteral administration, suggests minimal absorption from the gastrointestinal canal.

5. Aminotriazole (3-amino-1,2,4-triazole) is herbicidally active against numerous species of plants. After absorption, it is translocated through the root as well as through the foliage. It breaks down rather quickly in the soil, and the exposed plants become chlorotic, as aminotriazole seems to interfere with chlorophyll production (47).

In animal experiments, aminotriazole showed a low acute toxicity, the LD50 after oral administration having been found to be 14,700 mg/kg for mice (114) and about 25,000 mg/kg for rats (90). No signs of poisoning were seen after intravenous injection of 1750 mg/kg to a cat (90), of 1600 mg/kg to mice (90), or 1200 mg/kg to a dog (114). A single intraperitoneal dose of 4000 mg/kg was tolerated by mice, whereas 21 doses of 1000 mg/kg each to rats, distributed over 45 days, produced an increase of the thyroid weight of 328% in males and 410% in females, while at the same time the growth and intake of food were found to be normal (90).

In feeding experiments on rats extending over 68 weeks, no effect was seen on the growth or intake of food at 10 and 50 p.p.m. in the diet, as compared with

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controls, whereas 100 p.p.m. caused a decrease in growth and food intake in male rats during the last few experimental weeks. However, in male rats given 50 p.p.m. the thyroid became enlarged after 13 weeks (114). Investigations into the effect on the thyroids of rats of 2 years of feeding with aminotriazole are stated to have given the following results: In the control group, there was found one case of cystic follicle with papillary changes, while among the animals given 10 p.p.m. one out of ten examined presented adenoma; further, after 50 p.p.m. two out of 15 examined had adenoma and one apparently adenocarcinoma. Rats given 500 p.p.m. of aminotriazole in the diet for 17 weeks, which were then retained on a diet free of the compound for 2 weeks prior to sacrifice, appeared to have normal thyroids at the time of sacrifice (62).

The observation that rats fed with 100 p.p.m. of the compound for 2 years "developed a significant number of thyroid adenomas and adenocarcinomas," as well as the demonstration of residues of aminotriazole in marketed cranberries (31), caused prohibition of sale of cranberries and cranberry products of the 1958 and 1959 crops from certain parts of the United States. Furthermore, it resulted in an official announcement from the Secretary of Health, Education, and Welfare that the reason for the prohibition was "possible contamination by a chemical weedkiller, Aminotriazole, which causes cancer in the thyroid of rats" (37). In the ensuing discussion, doubts were raised as to whether the marked antithyroid action of aminotriazole can justly be characterized as carcinogenic (10, 62).

The mechanism of the antithyroid action of aminotriazole seems, on the basis of experiments on rats, to be identical with that of thiouracil derivatives (1a, 62). The absorption of I^{131} by the thyroid is depressed in both rats (1a) and humans (10), because its incorporation as organically bound iodine is obstructed (1a). Following injection of aminotriazole, a reversible inhibition has been noticed of the catalase activity in thyroid tissue (1a) as well as in kidney and liver tissues (49, 50, 104). However, to obtain a definite inhibition of the catalase activity, larger doses of aminotriazole are required than are necessary to depress the absorption of I^{131} by the thyroid (1a). Hence, inhibition of catalase activity can hardly account for the antithyroid action. Studies, *in vitro*, using purified catalase preparations from liver and red blood corpuscles, have under special experimental conditions shown irreversible inhibition (69). However, the results of experiments with thyroid tissue suggest that inhibition of thyroid peroxidase is the essential factor (2), a hypothesis that is borne out by the results of experiments with peroxidase from milk and vegetable tissue, which are likewise inhibited by aminotriazole (19). It should be added, however, that the question has not yet been clarified, since enzymes that are active in the purine metabolism of bacteria are also inhibited, in these cases reversibly by aminotriazole; this observation is utilized experimentally (84, 117).

IV. CONCLUSIONS

Apart from the fact that most information regarding the toxicology of the herbicides is as yet rather fragmentary—a situation which will be considerably improved when reports from the biological laboratories of industry become avail-

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able to the public—it is important that attention not be confined to the toxicity of the original compounds. Their influence on plant metabolism might, for instance, block synthetic chains, leading to the accumulation of endogenous toxic products in vegetables. In this respect it has been shown that 2,4-D, applied in subtoxic amounts to such plants as sugar beets, increases the concentration of nitrate to twenty times the normal level. Consequently, feeding with the foliage involves a risk of poisoning (99). Attention has also been focused on the increase of cyanide in cyanogenic plants exposed to the action of herbicides (103).

As to the toxicity of metabolites of the herbicides themselves, which are formed in the soil and plants after their distribution, little is known. The problem has been touched in connection with investigations into the breakdown of herbicides by the aid of the micro-organisms in the soil. In particular, the decomposition of the chlorinated phenoxy-acids and the chlorinated aliphatic acids by soil bacteria and fungi has been submitted to extensive studies (*vide* 11, 57, 58, 59, 60, 93, 121). It appears that the breakdown of these compounds is almost complete, involving dehalogenation (57–60) and hydroxylation of the side-chain (93). Therefore, a persistent deleterious effect on the microflora of the soil need not be feared after use of the great majority of these organic herbicides (38). This is probably due to the fact that in most cultivated soils the populations of bacteria and fungi are so rich that there will always be found at least a few individuals that can adapt to almost any substance presented to them. These will synthesize new specific enzyme systems using the foreign substance as substrate. The resistant strains of micro-organisms will therefore multiply excessively, until the "substrate" has been decomposed (11). In general, the biotransformation of herbicides in soil does not seem to lead to the production of more potent compounds (11, 93, 121). Certain inorganic herbicides, *e.g.*, chlorate, may, however, have a deleterious influence on bacterial nitrification in the soil. By reduction of chlorate, hypochlorite may be produced, which has a very strong inhibitory action on the growth of the soil bacteria (1). Pentachlorphenol may have a harmful effect, especially on the soil fungi; various emulsifying and surface-active agents in the herbicidal preparations may also interfere with the soil flora (38).

The fates of the herbicides in plants have as yet been scarcely elucidated, but intensive studies are being carried on to clarify biotransformation in plants. The explanation of selective phytotoxicity has been founded on these studies. Decisive progress was gained when it was discovered that the herbicidally inactive 4-(2,4-dichlorophenoxy)butyric acid is converted by *beta*-oxidation to the active 2,4-dichlorophenoxyacetic acid in plants where specific *beta*-oxidase systems are present. These plants are harmed while species not possessing the specific enzymes are resistant (109). Conversely, the triazine derivative, Simazin, in nonsensitive plants is degradated by the presence of different factors such as catalase, peroxidase, and polyphenols to substances without phytotoxicity (46a). Great difficulties in analytical procedures have to be solved before the fates of herbicides in plants are clarified (92). However, there is reason to expect that a more rational basis will eventually be available, both for the study of the metabolism of herbicides in the animal organism, and for the planning of experimental in-

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vestigations of the toxicity of the original compounds as well as of their metabolites. A knowledge of persistent metabolites in crops for consumption especially will be of very great importance in the study of long-term actions, including carcinogenic effects (35).

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