this experiment. The relationship of the ungular deformations to the administration of 2,4-D is dubious.

In a complementary experiment, a pregnant sow was fed 2,4-D at a dietary level of 500 p.p.m. during the entire pregnancy, and further on. During a protracted parturition, fifteen live and one stillborn piglet were delivered. The newborn piglets were underdeveloped and listless and showed a high mortality rate, ten out of fifteen dying within 24 hours. No malformations were observed. Autopsy findings in the dead piglets were those of a generalized anaemia. On continued feeding of 2,4-D at the same level to the survivors for another 6—7 months, growth depression was evident. On autopsy, no chracteristic gross changes were observed, apart from multiple disc degenerations in the cervical and lumbar spine in two animals. Heamatological, biochemical, and histopathological findings were similar to those observed in the preceding experiment, thus confirming the toxic action of the phenoxy acid. The dam developed weakness of the hind quarters and was slaughtered at 6 weeks *post partum* because of a complete inability to rise. Autopsy revealed excessive degenerative changes in various regions of the spine, which provided a histopathological explanation to the clinical signs. The toxicological significance of these changes, however, as well as of the spinal degeneration in the young pigs, remains obscure.

Rats. Ten pregnant albino rats, initially weighing about 350 g, were equally divided between an experimental and a control group. The experimental group received 2,4-D in the drinking water, at a level of 1000 p.p.m., during the gestation period and the following 10 months. The estimated daily dose of 2,4-D was 50—100 mg/kg body weight. No unequivocal clinical or morphological changes were observed during this period. On continued administration of 2,4-D to the second generation rats for up to 2 years, growth depression and a reduced intake of feed and water and also a slightly increased mortality, as compared with controls, were noticed. Clinical-chemical and histopathological examination did not reveal any definite abnormalities. This fairly high tolerance of rats to 2,4-D may be related to the high plasma disappearance rate of the phenoxy acid in this species (see Distribution, above), which most likely is the result of an effective renal excretion. In pigs a lower plasma disappearance rate was accompanied by a lower tolerance to 2,4-D.

Chickens. Six white Leghorn chickens of both sexes, originally weighing about 1 kg, were continuously fed 2,4-D at a dietary level of 500 p.p.m. Except for one bird dying after 5 months of renal gout, no distinct clinical

DOW 121537

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symptoms were seen. The survivors were sacrificed at intervals between one and 18 months. The autopsy findings were mostly of a nonspecific character such as waxy muscular degeneration, proventricular ulceration, peribronchitis, and generalized anaemia.

In another experiment, also extending over 18 months, sixty day-old

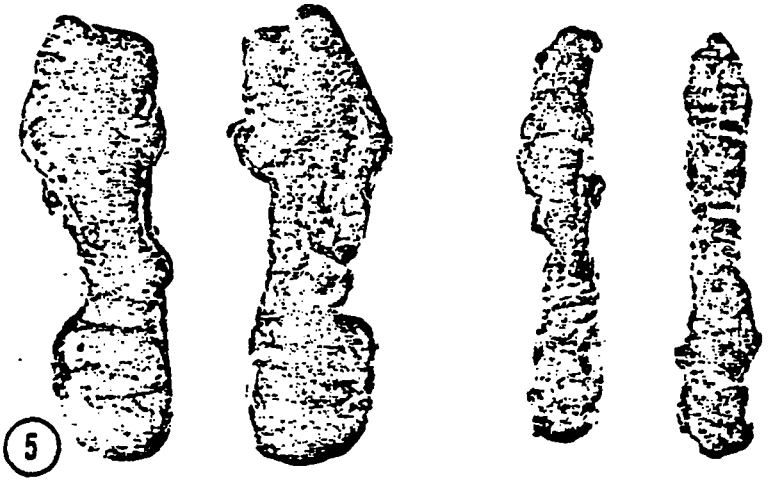




Fig. 5. Hypertrophic kidneys (left) of a 5-months old chick given 2,4-D amine (1000 p. p. m. in the drinking water) since 3 days old, as compared with normal kidneys (right) of control chick of the same age.

Fig. 6. Section of hypertrophic kidney. Tubular dilatation and hypertrophy. PAS, 300 ×.

Fig. 7. Section of normal chick kidney. Normal tubules. PAS, 300 ×.

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broiler chicks of both sexes were evenly divided between one experimental group, receiving 2,4-D amine (1000 p.p.m.) in the drinking water, and one control group. Apart from a reduced egg production in laying hens of the experimental group, no specific clinical signs were observed. Eleven treated and twelve control birds died or were killed because of disease during the



Fig. 8. Section of hypertrophic kidney. Glomerulus with extremely dilated capsular space. PAS, 300 ×.

Fig. 9. Section of normal chick kidney. Normal glomerulus. PAS, 300 ×.

DOW 121539

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experiment. The causes of death in both groups were mainly coccidiosis, leukosis, lymphomatosis, enteritis of unknown origin, or inanition of unknown origin. Survivors were sacrificed and autopsied at intervals between 2 and 18 months. The 2,4-D-treated birds, dead as well as killed, displayed a spectacular kidney enlargement due to epithelial proliferation, a finding which seems not to have been reported previously (Figs. 5—9).

The seemingly exclusive development in chickens of this lesion may possibly be related to the fact, that of the species examined this is the only one having a renal portal system which enables intestinally absorbed substances to pass directly to the kidney. Interestingly, the renal hypertrophy developed only in those chicks that had been continuously exposed since a few days old to the phenoxy acid. Apparently, this finding provides a parallell to the observation of *Carr* (1956), that only very young chicks are susceptible to leukaemia virus-induced renal carcinoma. In both instances the reactivity may be associated with the abundance of residual embryonic tissue in the young birds.

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Summary

The investigations summarized here aimed at developing methods for isolating, detecting and determining residues of chlorinated phenoxyaliphatic acids (phenoxy herbicides, "hormone weed killers") in biological materials, and at studying the distribution and toxicity of these materials in experimental animals.

After examining the conditions for isolation of phenoxy acids from different types of biological materials, an analytical method was adopted involving solvent extraction of the sample (the technique varying with the type of material), purification of the extract by partitioning between immiscible phases, and separation and identification of the acids by thin-layer chromatography. The eluted acids are determined photometrically.

The described method recovers phenoxyacetic acids from various biological materials to the extent of 70—90 per cent, with a standard deviation of 3—5 per cent (at the 4-p. p. m. level). The sensitivity is about 0.1 μg of phenoxy acid.

Applying this method, the distribution of phenoxy acids was studied in calves, pigs, rats, and chickens. After the administration of soluble salts of 2,4-D and of 2,4,5-T as single oral doses of 50—100 mg/kg body weight, the plasma concentrations rose to a peak within 2—7 hours and then declined to insignificant levels over a few days. The plasma half-lives ranged between 3 and 12 hours, depending on species. Oral administration of a 2,4-D ester resulted in considerably lower plasma levels of 2,4-D being attained (no ester was detectable in plasma or tissues). 2,4-D apparently distributed itself rather evenly over the body water, the highest levels being attained in the excretory organs (liver and kidneys). High levels were also found in ovaries, adrenals, lungs and myocardium. The levels found in brain were relatively low, indicating a slow passage of the blood—brain barrier. Placental transfer in swine apparently was less restricted. The disappearance rate from tissues was fairly high, the average tissue half-lives for 2,4-D ranging between 5 and 30 hours, depending on the species. The highest disappearance rate was consistently seen in rats and the lowest in pigs. Elimination evidently took place mainly be renal and, to a smaller extent, by biliary excretion of the unchanged phenoxy acid.

Repeated administration of subtoxic doses did not lead to excessive accumulation of phenoxy acid in any of the tissues examined. Except for a

DOW 121541

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moderate increase in tissue levels relative to plasma, the distribution pattern did not change on repeated intake. In pigs and chickens, the capacity for excreting phenoxy acids was seen to rise during prolonged exposure.

In acute toxicity studies, reversible toxic effects from 2,4-D amine were observed after single oral doses of 200 mg/kg in calves and of 100 mg/kg in pigs, respectively, doses above 500 mg/kg causing severe poisoning in pigs. Rats and chickens seemingly tolerated single doses of 100 and 300 mg/kg, respectively. On repeated oral administration, daily doses of 50 mg/kg could be toxic to pigs, whereas chickens tolerated 300 mg/kg/day for 2 weeks without outward effects.

The results suggest that clinical signs of acute or subacute poisoning are likely to appear, at least in pigs, if the plasma concentration of phenoxy acid exceeds a threshold value of about 200—300 μg/ml.

In long-term studies, signs of toxicity from 2,4-D appeared in pigs at a dietary level of 500 p. p. m. (corresponding to a daily intake of 25—50 mg/kg) and in chickens at 1000 p. p. m. given in the drinking water (equivalent to 50—100 mg/kg/day). In rats administration of 2,4-D in the drinking water at 1000 p. p. m. (50—100 mg/kg/day) over two generations caused a reduced weight gain and an increased mortality in the second generation. No unequivocal morphological changes were observed.

In the pig experiments, the main symptoms were growth depression, locomotory disturbances, anaemia and albuminuria. On autopsy, degenerative changes were seen in the liver and kidneys. On feeding 2,4-D at the level of 500 p. p. m. to a pregnant sow during the gestation period and for 6 further weeks, no characteristic signs were observed. Autopsy did not reveal any changes attributable to the intake of 2,4-D. The newborn piglets, however, were underdeveloped and exhibited a high mortality rate (66 per cent). Continued administration of 2,4-D to the survivors resulted in growth depression, persistent anaemia and degenerative changes in the liver and kidneys.

In the chicken experiments, a spectacular renal hypertrophy was the main finding, together with a reduced egg production. The renal lesion developed only in those birds that had been exposed to phenoxy acid since a few days old.

The chronic oral toxicity of 2,4-D to the species examined appears to be moderate. Apart from the notable nephrotoxicity demonstrated in chicks, the observed long-term effects seem to be non-specific. Attention should be paid to the high mortality rate in the newborn piglets and the reduced egg production in the chickens, however, which findings, together with the demonstrated accumulation of phenoxy acid in foetal tissues and in ovaries, might be indicative of a phenoxy acid-induced interference with reproduction.

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DOW 121543

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DOW 121544

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PHILADELPHIA, PA 19104

January 23, 1968

DOW 0012 025

Mr. V. K. Rowe
Biochemical Research Laboratory
1803 Building
The Dow Chemical Company
Midland, Michigan 48640

Dear V. K.:

This note is a follow-up to my report of July 8, 1966. In that study, you will recall that 6 groups of healthy adult subjects received small and increasing doses of tetrachlorodibenzo-p-dioxin. We followed a specific protocol laid down by you. Unfortunately, not a single subject developed acne nor was there any evidence of toxicity.

This encouraged me to proceed more vigorously. We then assembled a new panel of 10 subjects and applied 0.05 ml of a 1% solution in alcohol chloroform to a one inch square on the back. These applications were made every other day for one month. The treated sites were covered with a non-occlusive gauze square. Each week for 6 weeks, the following laboratory tests were done: Urinalysis, CBC, BUN, SGOT, Alkaline phosphatase, and Creatinine clearance.

Clearly, this exposure was immensely greater than the former one. 8 of 10 subjects showed acne form lesions usually beginning 3 to 4 weeks. This began as with a typical development of comedones. In 3 instances, the lesions progressed to inflammatory pustules and papules. These lesions lasted for 4 to 7 months, since no effort was made to speed healing by active treatment. Biopsies were obtained in 5 instances at various stages. The histologic and clinical manifestations were in every way comparable to classical chloracne. The lesions were indistinguishable from those obtained by Dow 6X and Halowax.

In no instance was there laboratory or clinical evidence of toxicity. The subjects remained well throughout the study.

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These results implement the conclusions formerly drawn, namely: it is much more difficult to induce acne in the human than in the rabbit ear. The process begins more slowly and the doses required are very much greater. However, unlike the rabbit ear, the comedones often become inflammatory. It is a certainty that the rabbit ear is exceptionally sensitive to acneigenic chemicals and is an excellent system for the detection of such chemicals. Finally, it may be said that chloracne closely mimics acne vulgaris. The only difference is the paucity of anaerobic organisms in the comedo. One may conclude that bacteria are not very significant in the pathogenesis of chloracne.

Very sincerely yours,

Albert M. Kligman, M. D.

AMK/klf

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Report

To

DOW CHEMICAL COMPANY

On

GROUP (1 Through 6)

DOW 749649

Submitted

By

Clover Laboratories, Inc.

Philadelphia, Pennsylvania 19101

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Code: F Forehead; B = Back; Mcg = Microgram GROU 1

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
1	22	185	N	0.2	1	0.2	R	12	9.5	11	8743	15.5	63 Cu/Min	15	10.0	11	9085	15.4	
1				0.1	2	0.2	F							12	10.4	13	9059	16.5	77 Cu/Min
2	29	210	N	0.2	1	0.2	F	16	12.8	10	9021	15.2	40 Cu/Min	16	11.6	12	9834	15.5	
2	Sub Dis	ject Charged																	
3	22	150	N	0.2	1	0.2	F	18	12.0	13	7405	15.6	81 Cu/Min	15	10.2	10	6573	15.4	
3				0.1	2	0.2	F							15	11.5	10	7803	14.7	64 Cu/Min
4	23	190	N	0.2	1	0.2	F		10.2	11	9981	13.5	47 Cu/Min	20	9.4	8	6003	12.6	
4				0.1	2	0.2	F								10.6	11	6390	12.8	54 Cu/Min
5	32	185	N	0.2	1	0.2	F	19	12.2	12	7705	14.3	74 Cu/Min	17	10.7	10	4570	13.8	
5	Sub Dis	ject Charged																	

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Code: Back; Mcg = Microgram

GROUP I

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY						FINAL LABORATORY					
								SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
6	28	139	N	0.2	1	0.2	B	20	8.8	10	9879	15.9	42 Cu/Min	16	8.9	10	6386	15.0	
6				0.1	2	0.2	B							19	8.5	10	7469	15.2	77 Cu/Min
7	25	238	N	0.2	1	0.2	B	17	9.3	9	6220	15.5	91 Cu/Min	16	10.5	14	7347	14.8	
7				0.1	2	0.2	B							17	12.1	13	7665	14.4	82 Cu/Min
8	27	156	N	0.2	1	0.2	B	20	7.7	12	9766	15.6	63 Cu/Min	20	7.6	12	9379	15.5	
8				0.1	2	0.2	B							18	8.2	14	9721	15.7	47 Cu/Min
9	26	191	N	0.2	1	0.2	B	15	9.5	11	8446	15.4	74 Cu/Min	19	9.8	11	9700	15.4	
9				0.1	2	0.2	B							17	10.0	10	9721	15.0	90 Cu/Min
10	24	225	N	0.2	1	0.2	B	20	12.0	14	7471	16.4	73 Cu/Min	16	11.3	14	7194	15.4	
10				0.1	2	0.2	B							18	11.7	12	9146	15.8	42 Cu/Min

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Code: 1 Forehead; Mcg = Microgram

GROUP 2

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
1	28	170	N	0.5	1	0.5	F	15	13.8	14	5932	14.8	63 Cu/Min	16	11.3	13	4446	14.5	
1				0.1	5	0.5	F							15	15.4	14	6915	14.2	54 Cu/Min
2	24	186	N	0.5	1	0.5	F	16	14.3	17	9134	15.7	97 Cu/Min	20	11.3	13	11908	14.7	
2				0.1	5	0.5	F							13	14.3	14	9904	14.7	67 Cu/Min
3	28	135	N	0.5	1	0.5	F	12	11.5	11	6843	15.7	47 Cu/Min	18	10.7	11	6329	14.5	
3	Subject Discharged													16					74 Cu/Min
4	25	154	N	0.5	1	0.5	F	17	13.6	11	7825	14.4	72 Cu/Min	14	11.4	12	5384	13.8	
4				0.1	5	0.5	F							20	13.6	10	8088	13.4	99 Cu/Min
5	35	156	W	0.5	1	0.5	F	17	13.1	9	6737	14.7	84 Cu/Min	14	11.3	13	6894	13.9	
5				0.1	5	0.5	F							18	12.3	10	9429	14.0	94 Cu/Min

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Code: P Back; Mcg = Microgram GROUP 2

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
6	23	175	N	0.5	1	0.5	B	18	11.5	9	7573	16.5	49 Cu/Min	14	12.3	8	5009	15.4	
6				0.1	5	0.5	B							17	12.3	9	7245	14.8	67 Cu/Min
7	26	130	N	0.5	1	0.5	B	15	9.7	13	6197	13.7	72 Cu/Min	15	10.2	12	6232	14.5	
7				0.1	5	0.5	B							16	10.0	12	7173	15.0	67 Cu/Min
8	28	150	N	0.5	1	0.5	B	20	8.8	12	7475	14.4	93 Cu/Min	15	8.7	16	5680	14.8	
8				0.1	5	0.5	B							18	9.1	13	7874	14.3	74 Cu/Min
9	22	138	N	0.5	1	0.5	B	12	10.9	13	7121	15.7	84 Cu/Min	14	9.4	11	5828	15.4	65 Cu/Min
9				0.1	5	0.5	B							17	10.0	12	8142	15.5	
10	32	163	N	0.5	1	0.5	B	20	10.6	11	6819	15.7	75 Cu/Min	18	10.2	14	6927	13.9	
10				0.1	5	0.5	B							14	91	11	9731	13.7	96 Cu/Min

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Code: = Forehead; Mcg = Microgram

GRO' 3

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
1	29	150	N	1.0	1	1.0	F	32	10.4	12	8155	15.5	69 Cu/Min	17	10.7	11	9346	15	
1				0.5	2	1.0	F							20	10.4	13	7429	14.5	87 Cu/Min
2	26	148	W	1.0	1	1.0	F	17	11.1	9	7690	13.2	81 Cu/Min	26	10.7	10	6657	12.0	
2				0.5	2	1.0	F							16	10.6	9	7405	12.4	72 Cu/Min
3		139	N	1.0	1	1.0	F	19	10.2	11	7726	14.6	97 Cu/Min	24	9.6	15	5481	15.0	
3				0.5	2	1.0	F							20	12.3	14	7440	14.3	74 Cu/Min
4	23	170	N	1.0	1	1.0	F	15	13.1	9	7003	13	99 Cu/Min	24	11.1	8	7757	12.8	
4				0.5	2	1.0	F							27	11.9	11	7906	12.2	97 Cu/Min
5	28	156	W	1.0	1	1.0	F	21	12.7	10	6357	12.8	67 Cu/Min	15	11.6	11	5210	12.5	
5				0.5	2	1.0	F							19	11.9	13	8121	12.0	73 Cu/Min

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Code: P = Back; Mcg = Microgram

GROUP 3

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
6	40	146	N	1.0	1	1.0	B	14	12.2	12	8507	13.6	82 Cu/Min	20	11.3	11	6414	12.5	
6				0.5	2	1.0	B							18	11.7	12	8911	12.5	99 Cu/Min
7	35	165	W	1.0	1	1.0	B	15	11.8	9	9061	11.5	81 Cu/Min	20	11.1	8	10212	15.2	
7	Sub Dis	ject charged																	
8	27	140	W	1.0	1	1.0	B	17	14	11	7233	14.7	71 Cu/Min	19	13.4	10	7433	15	
8				0.5	2	1.0	B							21	13.6	8	6929	14.5	83 Cu/Min
9	27	210	N	1.0	1	1.0	B	21	12.3	13	9943	15.9	97 Cu/Min	24	11.4	13	7946	15.5	
9				0.5	2	1.0	B							28	10.2	9	8610	15.3	75 Cu/Min
10	26	142	N	1.0	1	1.0	B	19	10.9	11	7036	15.2	84 Cu/Min	23	10	10	6600	14.3	
10				0.5	2	1.0	B							21	9.7	13	7124	15	73 Cu/Min

5560

0004332

Code: F = Forehead; Mcg = Microgram

GROU 4

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
1	32	193	N	2.0	1	2.0	F	24	13.0	10	4962	13.1	74 Cu/Min	19	13.E	11	7171	14.1	
1	Sub Dis	ject charged																	
2	21	199	N	2.0	1	2.0	F	18	10.1	10	8994	14.0	99 Cu/Min	36	22.4	11	8608	14.5	
2				0.5	4	2.0	F							15	16.2	11	9938	13.0	72 Cu/Min
3	24	200	N	2.0	1	2.0	F	16	13.0	11	8716	14.1	63 Cu/Min	26	16.9	12	8167	15.1	
3				0.5	4	2.0	F							17	13.9	10	9919	14.5	68 Cu/Min
4	27	170	N	2.0	1	2.0	F	16	9.9	10	8878	13.4	87 Cu/Min	16	10.6	9	6274	13.1	
4	Sub Dis	ject charged																	
5	28	150	N	2.0	1	2.0	F	17	8.7	12	5812	14.4	81 Cu/Min	20	11.0	10	8302	14.1	
5				0.5	4	2.0	F							32	9.4	11	7796	14.7	93 Cu/Min

0004338

5561

Code: B = Back; Microgram = Mcg GRO' 4

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
6	28	150	N	2.0	1	2.0	B	19	8.7	11	8764	15.4	74 Cu/Min	19	12.1	18	7085	14.8	
6				0.5	4	2.0	B							16	9.8	13	7881	15.5	93 Cu/Min
7	41	160	N	2.0	1	2.0	B	16	10.9	10	7151	12.5	63 Cu/Min	16	10.6	11	9739	13.5	
7	Sub Dis	ject charged																	
8	36	147	N	2.0	1	2.0	B	20	10.3	10	9074	13.0	84 Cu/Min	16	10.6	10	6841	13.5	
8	Sub Dis	ject charged																	
9	21	140	N	2.0	1	2.0	B	16	131	13	7991	13.0	54 Cu/Min	20	12.7	14	7829	13.0	
9				0.5	4	2.0	B							18	12.9	19	9811	13.5	78 Cu/Min
10	30	166	N	2.0	1	2.0	B	16	9.7	13	8243	13.0	69 Cu/Min	18	11.0	9	7357	13.5	
10				0.5	4	2.0	B							15	10.0	10	8583	13.5	85 Cu/Min

0004339

5562

Code: Forehead; Mcg = Microgram

GROUP 5

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
1	49	210	N	4.0	1	4.0	F	18	9.9	12	7729	12.9	83 Cu/Min	13	10.7	13	9978	14.0	
1				1.0	4	4.0	F							16	11.0	12	9941	14.6	72 Cu/Min
2	30	210	N	4.0	1	4.0	F	25	8.4	11	8312	15.5	62 Cu/Min	18	9.9	10	9947	15.1	
2	Sub Dis	ject charged																	
3	23	165	N	4.0	1	4.0	F	16	7.7	9	9982	15.1	90 Cu/Min	12	9.2	11	8873	14.5	
3				1.0	4	4.0	F							14	9.7	10	7781	14.9	77 Cu/Min
4	23	170	N	4.0	1	4.0	F	14	6.2	10	9764	15.2	76 Cu/Min	12	7.9	11	9367	15.1	
4				1.0	4	4.0	F							11	8.1	12	9074	14.8	91 Cu/Min
5	Sub Dis	ject charged																	
5	Sub Dis	ject charged																	

5563

CC04310

05951

Code: [illegible] Back; Mcg = Microgram

GR01 5

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY SGOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
6	31	152	N	4.0	1	4.0	B	19	10.7	10	5043	12.0	89 Cu/Min	16	9.7	10	7378	13.4	
6				1.0	4	4.0	B							15	11.3	11	7687	13.1	80 Cu/Min
7	44	187	N	4.0	1	4.0	B	22	11.6	10	9941	14.4	77 Cu/Min	13	13.5	10	7378	13.4	
7				1.0	4	4.0	B							18	13.0	10	12504	14.7	62 Cu/Min
8	43	202	N	4.0	1	4.0	B	19	12.2	17	7781	14.3	91 Cu/Min	16	12.0	10	7853	15.1	
8	Sub Dis	ject charged																	
9	24	157	N	4.0	1	4.0	B	19	9.2	8	7465	13.9	90 Cu/Min	16	9.9	10	7415	13.6	
9				1.0	4	4.0	B							19	12.0	10	8948	13.1	80 Cu/Min
10	Sub Dis	ject charged																	
10	Sub Dis	ject charged																	

5564

0004341

Code: Forehead; Mcg = Microgram

GRO' 6

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY 3GOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY 3GOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
1	29	166	N	8.0	1	8.0	F	15	11.8	11	9537	14.1	93 Cu/Min	16	10.3	12	7391	13.7	
1				1.0	8	8.0	F							15	10.5	13	7446	13.9	81 Cu/Min
2	22	164	N	8.0	1	8.0	F	12	9.5	14	9524	16.4	72 Cu/Min	16	8.4	12	5695	15.0	
2				1.0	8	8.0	F							16	8.0	11	5521	14.8	87 Cu/Min
3	Sub Dis	ject charged																	
3	Sub Dis	ject charged																	
4	38	201	W	8.0	1	8.0	F	13	11.1	15	8367	150	99 Cu/Min	19	9.7	10	6591	15.2	
4				1.0	8	8.0	F							17	9.5	18	6761	15.8	72 Cu/Min
5	33	169	W	8.0	1	8.0	F	11	10.2	18	9986	15.2	68 Cu/Min	20	9.5	13	9437	13.8	
5	Sub Dis	ject charged																	

5565

C004342

Code: B - Back; Mcg = Microgram

GROUP 6

Subj. #	Age	Weight	Race	Mcg Daily Dose	No. Dose	Total Dose	Site	INITIAL LABORATORY 3GOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.	FINAL LABORATORY 3GOT	Alk Phos	BUN	WBC	Hb.	Creat. Cl.
6	38	201	W	8.0	1	8.0	B	28	12.5	11	8517	15.9	94 Cu/Min	30	12.0	13	6796	13.3	
6				1.0	8	8.0	B							22	10.5	10	7248	13.6	87 Cu/Min
7	Sub Dis	ject charged																	
7	Sub Dis	ject charged																	
8	25	154	N	8.0	1	8.0	B	14	9.7	11	8027	15.6	99 Cu/Min	18	8.8	12	5469	14.1	
8	Sub Dis	ject charged																	
9	33	250	N	8.0	1	8.0	B	22	11.9	10	10969	17.0	97 Cu/Min	37	13.5	12	6081	16.0	
9				1.0	8	8.0	B							20	12.0	13	6913	15.4	73 Cu/Min
10	22	134	W	8.0	1	8.0	B	18	10.6	13	7822	16.0	91 Cu/Min	26	18.8	11	6003	15.1	
10	Sub Dis	ject charged																	

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National Clearinghouse
for
Poison Control Centers

Bulletin

...ch - April 1965

U.S. DEPARTMENT OF
HEALTH, EDUCATION AND WELFARE
Public Health Service
Washington D.C. 20201

DOW M 25803
MN 073280

DEATHS FROM CHLORINATED PHENOXYACETIC ACIDS
(2,4-D; 2,4,5-T; MCPA)

The most widely used herbicides are 2,4-D, 2,4,5-T; and related compounds. Whether or not these are actually toxic is a matter of controversy. --Rachel Carson.

There may not be controversy when these chemicals are used as directed around the household or in agriculture, but the Michigan farmer who developed peripheral neuropathy four days after he repeatedly used his bare hands to unplug a tractor-towed automatic sprayer might dispute this. 2,4,5-T was implicated recently with poison oak in the illness of two sisters who played for several hours in a yard which had been sprayed heavily a short time before. The next day both girls had a generalized erythema of the skin, minimal edematous swelling of the oral and vaginal mucous membranes, slight swelling of lips and eyelids, inflammation of the mouth, albuminuria and oliguria.

Upon receipt of a fatal case history due to suicide in a 46-year-old Connecticut man, a review of the Clearinghouse's reports was made. In the past three years, the Clearinghouse has received several reports of peripheral neuritis and skin rash following exposure to these products, in addition to those already cited.

Other symptoms that were associated with the chlorinated phenoxyacetic acid derivatives were: weakness of chest muscles associated with pneumonia and hematuria; lethargy with poor muscle tone; two brothers with ataxia, weakness and urinary frequency; irritability, lethargy; increase or decrease in temperatures. Most of the victims that had symptoms had vomited. There was one report of hallucinations after inhalation of 2,4,5-T.

2,4-D and its derivatives serve as a base material from which soluble esters and salts are produced. The LD50 of these products range from 300 to 700 milligrams per kilogram orally.

When the chlorophenoxy herbicides are ingested in single large doses they may produce death. Four cases have been described in the literature and a 5th case has been reported to the Clearinghouse. A Connecticut truck driver in a suicide attempt ingested a cupful of weed killer which was approximately 20% 2,4-D and 40% 2,4,5-T. Within an hour, he became nauseated and began to vomit. Approximately one hour after the ingestion he was found in a chair conscious but in a dazed condition. He was admitted to the hospital an hour and a half later, still conscious, but disoriented.

On his second hospital day he was on the hospital critical list because his general condition was getting worse. His temperature was 104° F., his blood pressure 70/50, and although his urinary output was described as good, his BUN had risen from 16 milligrams percent to 48. He was hyperventilating and had basalar rales.

Late in the second day of hospitalization the patient's blood pressure was very low despite large doses of Aramine and a trial on Levophed. He had sinus tachycardia (150), a central venous pressure of minus 3, temperature of 104° F, hyperventilation, blood PH of 7.43, and anuria. He also had a generalized erythema.

The patient developed scattered crepitant rales in the right lung and died of cardiac standstill (no ventricular arrhythmias were noticed) early in the morning of his third hospital day, approximately 46 hours after the ingestion.

This case had several similarities to the case report of poisoning by MCPA (2 methyl-4-chloro-phenoxyacetic acid). Vomiting occurring shortly after ingestion and unconsciousness appearing in a few hours. Both had lowered blood pressure but in the MCPA case it could be maintained with metaraminal. However, none of the neurological symptoms (facial twitching, constricted pupils, decreased tendon reflexes, clonic spasm of limbs) were mentioned in the present case.

Early deaths in animals by large doses of chlorinated phenoxyacetic acid derivatives were attributed to ventricular fibrillation. In delayed deaths, there was a disinclination to move, progressing to rigidity of skeletal muscles (myotonia) and ataxia. The severe cases had progressive apathy, muscular weakness of the hind limbs, paralysis, clonic spasms and finally coma.

DOW 0004807

Ingestions of these herbicide[illegible] careful gastric lavage and symptomatic and supportive therapy. Clinical Toxicology of Commercial Products recommends Quinidine Sulfate [illegible] 0.2 grams orally every two hours for the first [illegible] hyotonia, if present, and to suppress ventricular [illegible] Severe cases may require cardiac monitoring with defibrillator [illegible] attached to the chest. Fever should be treated using physical [illegible] packs and alcohol sponges) since antipyretics may be [illegible] indicated.

DOW H 734505

Parke - Davis PARCODE

Parke-Davis has recently initiated a numerical product identification system known as PARCODE. The special number designation identifies each capsule, Kapseal, tablet and Emplet. The initials P-D identifies the product as a Parke-Davis preparation and the imprinted number identifies the particular product. Although the company's manufactured products will henceforth carry a PARCODE identification symbol, there may be considerable time required for utilization of the non-coded products currently in stock.

Coding or identification, have already been established by Eli Lilly and Company and the Norwich Pharmacal Company. The poison control centers have been sent a copy of the Parke-Davis recognition code and will be informed if any other pharmaceutical companies adopt similar systems.

ACUTE OVERDOSAGE OF DORIDEN

The CIBA Pharmaceutical Company has recently published a booklet on acute overdosage of Doriden (Glutethimide). It includes the recommended supportive care, the indications for dialysis, collection of specimens for laboratory examination, a commentary on acute overdosage as well as bibliographies. A copy of this booklet may be obtained by writing to the Medical Communications Section, CIBA Pharmaceutical Company, Summit, New Jersey 07901.

0004608

4/

DEFEROXAMINE

DOW A 754506

For several years the Ciba Corporation has been conducting research on the use of deferoxamine (Desferal), older name desferrioxamine, in the treatment of iron poisoning. Because there have been several articles published in the medical literature on its effectiveness, there has been a series of requests for this medication when poisonings have occurred. Since this drug was under investigation, it was only available to the authorized investigators. The list of investigators, willing to accept referrals of iron intoxications, could not be published. Therefore, the National Clearinghouse initiated a service to the Poison Control Centers to provide the nearest investigator's name and telephone number when an inquiry was made for the immediate treatment of an individual case. We felt this might be of benefit to a seriously ill patient, as well as to the investigator, by providing him a larger number of observations from which to judge the usefulness of this medication. This drug has been released for general medical use, and until the Manufacturer can complete its distribution this service will be continued.

Deferoxamine, a chelating agent, complexes with iron to form ferrioxamine which prevents the iron from entering into further chemical reactions. The chelate is soluble in water, is excreted by the kidney, and produces a characteristic reddish color to the urine. Theoretically, deferoxamine will bind 8.5% of its weight of ferric iron. Thus, the initial recommended dose of 1 gram would bind 85 milligrams of ferric iron and the maximal 24 - hour dosage of 6 grams would bind over one-half gram of iron, the equivalent of 2 1/2 grams of $FeSO_4 \cdot 7H_2O$.

The manufacturer has offered to supply for distribution package inserts on its use and cautions to all of the poison control centers.

Henry L. Verhulst, Director

John J. Crotty, M.D., Associate Director

C0041609

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REPORT NUMBER
GH-P 551

BIOPRODUCTS DEPARTMENT

DATE
October 21, 1968

AUTHORS
Marguerite L. Long

TITLE
Review on the Metabolism of Phenoxy Compounds in Plants and Animals

INFORMATIVE SUMMARY WITH CONCLUSIONS

This extensive review was undertaken to determine whether phenoxy herbicides undergo changes in plants or animals which would result in significant residues of metabolites which would have to be accounted for in the establishment of tolerances by FDA. The review is divided into sections as outlined in the attached Table of Contents.

The metabolic products shown to be formed in plants were short-lived intermediates whose nature depended on the chemical structure of the phenoxy compound, the rate and mode of application of the herbicide, the stage of growth and metabolic condition of the plants and, most importantly, on the species and varieties of the plants studied. In spite of very extensive work during the past twenty years, no one has been able to identify the products, although hydroxylation of the ring is believed to occur in some plants. In animals, each compound was excreted unchanged, virtually quantitatively within a short time. Traces of residues were found for only two or three days in milk from cows maintained in pastures during and following treatment with 2,4-D.

It was concluded that there is no reasonable expectation that residues of metabolites of the phenoxy herbicides could be present in significant amounts in raw agricultural commodities of plant or animal origin as a result of currently registered uses for 2,4-D, 2,4,5-T, silvex or MCPA. (This review was prepared for the NACA as part of a supplement to pesticide petitions for negligible tolerances of those phenoxy compounds in a number of crops. The total compilation included copies of the 73 references cited herein).

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5573

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DOW763479

5574

0004347

REVIEW ON THE METABOLISM OF PHENOXY COMPOUNDS IN PLANTS AND ANIMALS

Table of Contents

DOW763480

Summary Statement

Glossary of Terms

Review of the Literature

I. Metabolism in Plants

- A. Introduction
- B. Tabulation of Conditions Used in Studies with Plants
- C. Metabolism of 2,4-D
  1. Hydrolysis of Esters and Salts of the Herbicide
  2. Decarboxylation and Formation of the Phenol
  3. Conjugation into Water Soluble Detoxication Products
  4. Transformation into a Different Compound
  5. Beta-oxidation of the Side Chain in 4-(2,4-DB)
- D. Metabolism of 2,4,5-T and 2,4,5-TB
- E. Metabolism of MCPA and MCPB
- F. Metabolism of Silvex (2,4,5-TP)

II. Metabolism in Animals

III. Recent Residue Studies by Newer Methods

IV. Significance of Metabolites as Potential Residues in Foods and Feed

V. References

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C004348

TABLE 30.—*Summary of dosages of various organic herbicides that cause significant weight loss, reduced weight gain, or poisoning in cattle, sheep, and chickens* [1]

Herbicide	Dosage rate	Least number of dosages for—		
		Cattle	Sheep	Chickens
	Mg./kg.			
Chlorophenoxy compounds:				
2,4-Dichlorophenoxyacetic acid (2,4-D), alkanolamine salts (of the ethanol and isopropanol series).	500	--------	7	10
	250	1	10	10
	200	25	----------	----------
	100	86	8	----------
2,4-Dichlorophenoxyacetic acid (2,4-D), propylene glycol butyl ether ester.	250	3	2	4
	100	----------	----------	10
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), propylene glycol butyl ether ester.	500	----------	----------	5
	250	4	4	10
	100	--------	3	----------
2-(2,4,5-Trichlorophenoxy)-propionic acid (silvex), propylene glycol butyl ether ester.	250	--------	5	10
	100	19	9	10
2-Methyl-4-chlorophenoxyacetic acid (MCPA), alkanolamine salts (of the ethanol and isopropanol series).	500	8	--------	1
	250	--------	6	10
	100	--------	4	----------
Amide compounds:				
N,N-Dimethyl-2,2-diphenylacetamide (diphenamid).	500	----------	----------	10
	250	3	7	10
2-Chloro-*N,N*-diallylacetamide (CDAA).	250	--------	1	----------
	100	--------	1	1
	50	1	1	9
	25	1	4	10
2-Chloro-*N,N*-diallylacetamide (CDAA) and trichlorobenzyl chloride (TCBC).	250	--------	1	1
	100	1	1	10
	50	2	2	----------
	25	--------	5	----------
Phenyl urea compounds:				
3-(*p*-Chlorophenyl)-1,1-dimethylurea (monuron).	500	1	----------	----------
	250	--------	2	6
	100	--------	4	8
	50	----------	----------	10
	25	----------	----------	10
3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea (linuron).	250	--------	3	----------
	100	6	4	8
	50	10	1	10
	25	----------	----------	10
	10	----------	----------	10
3-Phenyl-1,1-dimethylurea (fenuron).	500	2	3	10
	250	--------	10	----------
	100	--------	8	----------
3-(3,4-Dichlorophenyl)-1,1-dimethylurea (diuron).	250	--------	1	8
	100	10	2	10
	50	----------	----------	10
	25	----------	----------	10
	10	----------	----------	10
Thiocarbamate compounds:				
S-2,3-Dichloroallyl diisopropylthiocarbamate (diallate).	250	--------	1	7
	175	----------	----------	10
	150	----------	----------	10
	125	----------	----------	10
	100	--------	3	----------
	50	3	3	----------
	25	1	1	----------
S-2,3,3-Trichloroallyl diisopropylthiocarbamate (triallate).	250	--------	2	7
	200	----------	----------	10
	175	----------	----------	10
	100	--------	1	----------
	50	3	2	----------
	25	5	----------	----------

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STATENS VETERINÄRMEDICINSKA ANSTALT
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Chemistry Department

December 18, 1969.

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Marguerite L. Leng, Ph.D.
Registration Section,
Agricultural Department,
The Dow Chemical Company,
P.O. Box 1706
MIDLAND, Michigan USA

Dear Dr. Leng,

Thanking you for your letter of December 11, 1969, I am enclosing some reprints including those you requested. Also included is a table of tissue distribution data for 2,4-D and 2,4,5-T in chickens, taken from a forthcoming paper (N.-E. Björklund and K. Erne, Acta Vet. Scand. vol. ~~11, 1970~~ 12, 243-256, 1971).

In a physiological study under way we have found chlorophenoxyacetic acids to be excreted in chickens by the proximal convoluted tubules in essentially unmodified form (acid-hydrolyzable conjugates making up less than 10 % of the excreted phenoxy acid) and apparently by a transport mechanism in common with uric acid and a few other aromatic acidic compounds (K. Erne and I. Sperber, to be published). 2,4,5-T was found to exhibit a higher affinity for the carrier than did 2,4-D.

I should greatly appreciate if you could let me have one copy of your finished review.

Sincerely yours,

K. Erne
Ph.D. (Vet. Sci.), Assoc. Prof.

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N.E. Björklund & K. Erne
Acta Vet. Scand. 1974

Table 2. Phenoxy acid levels in tissues of broiler chickens fed 2,4-D or 2,4,5-T at 1,000 p.p.m. in the drinking water for 104 days.

Chicken No.	Phenoxy acid	Sex	Tissue levels, µg/g, fresh weight					
			Liver	Kidney	Lung	Stomachs	Small intestine	Eggs (yolk)
C 2184/67	2,4-D	♂	4.0	5.0	3.6	20.0	10.0	-
C 2185/67	2,4-D	♀	6.3	10.0	7.2	28.0	15.0	0.4 - 2.0
C 2182/67	2,4,5-T	♀	3.5	8.5	8.0	65	18.0	0.8 - 2.1
C 2183/67	2,4,5-T	♀	2.0	3.3	4.1	-	-	0.7 - 1.6

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Bashirov, A. A., 1971.

...ces which were replaced by aqueous suspensions and ...equent replacement of the organochlorine compounds with ...ganophosphorus ones. These efforts included also an ...ducational campaign on the veterinary and medical, etc. ...pects of pesticide application. The first reassuring results of ...ch efforts are now available. The need in 1967 for chemical ...eatment of the potato crops, however, precluded achieving ...e desired results in preventing residue accumulation. In ...966, residues were found in 12.5% of sugar beet samples, ...5% of potato haulms and 19.4% of food product samples. In ...67, their corresponding values decreased to 6.71 and 6.8 [sic]. In 1967 as compared to 1966, there was a significant ...op in the number of mass pesticide poisonings of animals. ...terinary specialists played no little part in achieving these ...oals.

71-0507. Birbin, S. S.; Alekseeva, A. A.; Bulatov, A. A. (Tatishchevo District Veterinary Station of Saratov Province, Tatishchevo, USSR). The poisoning of swine treated with Granosan. *Veterinariya No. 8: 60-1; 1968.* (Russian)

Mass poisoning of swine with the organomercury ...rbicide Granosan was seen on two farms in Saratov Province. On the "October" Collective Farm in Tatishchevo District, ...3 of 414 swine of various ages were affected during August [1967], the acute period; 121 died and 145 in the agonal state had to be slaughtered. By November, another 44 animals had ...ed. On the "Fedorov" State Farm in Marx District, 211 of ...44 swine were affected. The cause of the toxicosis was the ...olonged feeding (up to 30 days) of ground barley that had ...en treated with Granosan and of granary grain sweepings ...hich contained a large percentage of the preparation. The ...st signs of poisoning appeared in suckling pigs and fatling ...ts 20 to 25 days after beginning feeding on the treated grain; ...n sows, the signs appeared in 30 to 40 days. At first, the ...imals refused food and water and became restless. There was ...me nasal mucous secretion. Then weakness in the hind limbs ...peared, with different types of movement coordination ...order. Some animals showed spinal involvement. Signs of ...ural disorders were quite clear, including muscular tremors, ...nvulsional jerking of the extremities and tetanic contrac...ns of the pelvic musculature. As their conditions worsened, ...e animals lay on their stomachs or sides, developing varying ...grees of paralysis with loss of pain sensation, rapid ...athing, etc. The younger swine almost all died within 3 to 6 ...s after the symptoms started; the sows' condition persisted ...ger until death (6 to 11 days) and 40 to 45% of the ...ected animals died. In some instances, the swine suffered ...above symptoms, including partial or complete loss of ...m, for 2 to 3 months. The autopsy findings were initially ...same in all the animals, the most constant changes being ...ed in the intestines. The intestinal mucous membrane was ...ered with dryish, dirty yellow or brownish-green deposits ...nected to the underlying tissue. Liver and kidney findings ...also described. Therapy with unithiol, cardiac agents, ...cillin and glucose were all ineffective. The fact of a delay ...the appearance of symptoms following Granosan intoxica... must be taken into account in diagnosing organomercury ...nings. The clinical symptoms and pathologicoanatomical ...ges in mass poisonings of swine with Granosan to a great extent recall the course of infectious diseases such as plague, Aujeszky's disease and paratyphoid, so that mercury poisoning should be eliminated during a differential diagnosis.

71-0508. Rusin, K. N.; Tokarev, N. F.; [Aroshenko, N. M. (Min. of State Farms, Ukrainian SSR, USSR). Cases of swine poisoning with Granosan. *Veterinariya No. 9: 61-3; 1969.* (Russian)

Cases of severe Granosan poisoning in swine are reported. In the fall of 1967 on the "Victory" State Farm in Nikolaev Province, all the Granosan-treated wheat not used in sowing was stored next to forage grain. The treated, thrashed grain was used as swine feed from November 11, 1967 at 2.2 kg/day. The contaminated grain was fed up to 26 days, since the initial poisoning symptoms were lacking. At 1 month, the 2- to 5-month-old piglets began to display clinical signs: refusal of food, depression, muscular tremors, etc. The oral mucosa was hyperemic; the feces were of a yellow color and in many cases liquid, covered with a thick, elastic coating, etc. Some swine had temperatures below normal for 5 to 6 days and, as a rule, these animals died (76% of those affected). The autopsy findings included marked plethora, hyperemic oral mucosa, enlarged lymphatics, uneven coloring of the lungs from grey-red to dark red which were of a doughy consistency, etc. The diagnosis was based on the pathologicoanatomical findings, the clinical picture and laboratory analysis, all of which excluded infectious disease. Mercury was found in samples of feed and of the parenchymatous organs. The treated grain, for example, contained 0.18 to 21.7 mg/kg Granosan. The therapeutic regimen, once the diagnosis was established and the feed changed, included glucose and caffeine i.v. and various doses of unithiol s.c. and i.m. But only 34% of the affected swine were saved. Follow-up over the next 5 months showed that poisoned swine were retarded in weight and the mercury salts also had a negative effect on sow productivity (premature, stillborn and aborted young). As a result of this incident, the "Victory" State Farm was not allowed to supply animal husbandry products to the market for 5 months.

71-0509. Bashirov, A. A. (Azerbaijani Res. Inst. of Labor Hygiene and Occupational Diseases, Baku, Azerbaijani SSR, USSR). The state of health in workers manufacturing the herbicides, the amine salt and the butyl ester of 2,4-D acid. *Vrachebnoe Delo No. 10: 92-5; 1969.* (Russian)

A medical examination was done of 292 persons engaged in the manufacture of the title herbicides; 248 were men and 44 women. Their ages ranged from under 20 (nine) to over 40 (19). Professionally, the workers' jobs ranged from laboratory worker to machinery operator; their work exposure ranged from under 5 years (194) to 6 to 10 years (98). The workers' general complaints included general weakness, rapid fatigability, frequent headaches of a dull nature mainly localized in the frontal-parietal area which intensified at the end of the work day (63%), vertigo (33%), etc. Objectively, arterial hypotension was found in 59 persons (20.2%). Frequent complaints were noted in 51.7% of those examined which were typical of digestive function disorders: dyspepsia, poor

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Health Aspects of Pesticides 4:139-140, 1971

appetite, radiating pains in the right epigastrium, etc. A slight increase in the liver was found in 12 persons. From these findings, 50 workers (41 men and 9 women) were selected for detailed clinical studies of the stomach and liver as well as of the cardiovascular system. Their case histories showed no particular diseases prior to their present work with the 2,4-D acid herbicides. A control group of 20 persons with no toxic chemical contact was included. Bradycardia was found in 20% which varied from a lying to a standing position. Arterial oscillography demonstrated that the mean dynamic pressure while lying was within normal limits but while standing this index showed asymmetry. Fourteen of the 50 persons examined had a lowered acid-forming function in the stomach; in eight there was a histamine-resistant achylia; etc. Liver dysfunction was often found: 22 of the 50 (versus four of the 20 controls) had disordered antitoxic function expressed as a decreased hippuric acid excretion in the urine. Liver protein-forming function was also disordered in 19 of the 50: lowered albumin level, etc. Four of the workers had a lowered prothrombin index. Blood sugar was lower than 70 mg% in 16 (versus two of the controls). The various liver dysfunctions found were significantly more pronounced in workers with longer work exposure to the herbicides. The results of the survey show that in the manufacture of the title herbicides, various disorders of the cardiovascular system and of the organs of digestion may occur. They point up the need for checking the cardiovascular system and the digestive organs in those entering work and in those already working in the production of 2,4-D herbicides.

71-0510. Lobzin, V. S. TSinovoi, P. E. (Dept. of Nervous Diseases, Kirov Military Medicine Acad., Leningrad, USSR). Neurological disorders in chlorophos poisoning. *Zh. Nevropatol. i Psikhiatr. 69(5): 679-83; 1969.* (14 references) (Russian)

Neurological disorders in acute chlorophos poisoning are described based on 41 case histories, 11 of which were severe (two patients died), 14 of median and 16 of light intoxication. The severity and character of the clinical signs depended on the dose and route of the poison into the body. In most instances, chlorophos had been taken internally. In the acute poisoning phase, the predominant symptoms were central nervous system lesion and vegetative disorders. The frequency of individual symptoms (muscular weakness, disorder of consciousness, headache and vertigo, tremor, delirium and hallucinations, sleep disorder, etc.) is shown in a table. Two individual case histories are detailed, illustrative of severe chlorophos poisoning. Both patients had respiratory disorder requiring intubation and were comatose. In one case, a 52-year-old man, the poisoning was so severe that in spite of various therapeutic measures (atropine i.v., gastric lavage, etc.) the patient died of respiratory and cardiac arrest. In the second case, a 46-year-old man, 3 days after treatment with i.m. TMB-4, i.v. atropine and glucose and regaining consciousness, the patient developed a delirious syndrome. After successful therapy for this, he complained of muscular pains and difficulty in walking. Objectively, decreased left knee and Achilles reflexes were noted; clumsiness in the fingers appeared. A diagnosis of toxic polyneuritis was made. The patient developed intense pains in the distal parts of the extremities. After 5 months of therapy, only slight improvement occurred. The movement disorders and disorder of sensitivity in the distal parts of the extremities proved very resistant to treatment. Of the 41 chlorophos poisoning cases, eight developed toxic polyneuritis; the latter appeared unexpectedly and anywhere from 10 days to 1½ months after the poisoning. Muscular atrophy was detected usually 3 to 4 weeks after the first signs of polyneuritis. A discussion of the problems of therapy is included.

See also 71-0439 71-0444 71-0445 71-0447 71-0451 71-0474 71-0489 71-0520 71-0526 71-0527 71-0535 71-0562 71-0569 71-0573 71-0580 71-0583 71-0605

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Residue Reviews 26, 37-62 (1969)

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Toxicity and hazards to man, domestic animals, and wildlife from some commonly used auxin herbicides

By
J. M. Way*

Contents

I. Introduction 37
II. Toxicity and hazards to man 38
III. Toxicity and hazards to domestic and laboratory animals 41
a) Acute and chronic toxicity 41
b) Indirect toxicity 46
IV. Bees and other insects 48
V. Soil animals 49
VI. Fish and aquatic organisms 50
VII. Wildlife in general 53
Summary 54
Résumé 55
Zusammenfassung 56
References 58

I. Introduction

This review has been compiled from searches in the literature for references to the toxicity and other hazards that may arise from the use of auxin herbicides, and specifically of formulations of MCPA (4-chloro-2-methylphenoxyacetic acid), S.S. 2,4-D (2,4-dichlorophenoxyacetic acid) and of their butyric acid analogues MCPB and 2,4-DB, of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), mecoprop [± α-(4-chloro-2-methylphenoxy) propionic acid], and 2,3,6-TBA (2,3,6-trichlorobenzoic acid).

Assuming 70 percent of the eight million acres of cereals grown in the United Kingdom to be treated annually with herbicides (Wood-

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ford 1964), it is apparent that the tonnage (circa 2,500 tons) of the compounds mainly used for this purpose is similar to the total tonnage of insecticides and fungicides used annually on all crops in the U.K. (circa 2,660 tons) (Strickland 1966). In 1962, Dadd (1962), from a survey of eastern England, found that over 90 percent of cereal fields treated with herbicides were treated with auxin compounds based on chlorinated phenoxy acid derivatives, notably salts of MCPA and to a lesser extent of mecoprop. Although the use of straight formulations of MCPA has been gradually declining (Woodford 1964) in favour of mixtures of compounds, many of these mixtures still include MCPA or other phenoxy compounds and increasingly include 2,3,6-TBA. In addition to its agricultural use on arable and pasture crops, 2,4-D together with 2,4,5-T, is used, or is likely to be used on an increasing scale, for scrub control and control of unwanted broad-leaved tree species in conifer plantations. 2,4-D is also used to a limited extent for control of submerged aquatic weeds. On a world-wide basis 2,4-D is probably used to a greater extent than any other herbicide and this is reflected in the greater number of references to this compound. The extensive use of a single biologically active family of compounds clearly presents a considerable potential hazard in respect to their direct and indirect toxicities to animals including man. It is a measure of the comparative safety of the auxin herbicides that, in spite of their increasing use throughout the U.K. since the mid-1940's, they continue to be applied much in the same routine way as are inorganic fertilizers, and with as little regard for any possible toxic hazard.

During the many years of their use throughout the world there have been notably few authenticated incidents of poisoning of domestic animals or wildlife resulting from the proper application of these compounds. The fact that fully authenticated incidents of poisoning have not been reported cannot be taken as an absolute guarantee of safety, particularly with regard to indirect or chronic toxicity, because of the frequent difficulties of attributing the underlying cause of an illness or of death, especially in wild animals. Nevertheless all the direct and circumstantial evidence to hand at the present time seems to indicate that incidents of poisoning have been extremely few and generally under exceptional circumstances.

II. Toxicity and hazards to man

The principal routes of toxicity to man are either orally or by inhalation; there appears to be little hazard of transport through the skin although individual allergies can develop leading to dermatitis (Vallet 1965). Eyes may be directly but are usually only temporarily affected. Hazards to man may occur from the concentrated chemical before dilution, from inhalation of spray or dust during application, or from ingestion of the chemicals in food or in water. Because the great-

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est hazards are from the concentrated chemical and because man is handling the chemicals in this form at all stages from manufacture to dilution, it follows that he is at greater potential risk than any other organism. However, there are very few reports in the literature of tests or incidents of poisoning of man by these compounds; the majority of these reports refer to accidental poisoning of children. As a result it is now generally accepted that auxin-type herbicides do not present a direct toxicity hazard to man (BARNES 1965) when correctly handled or used for weed control.

KRAUS in 1945 (in KEPHART 1945) reported that he had taken 0.5 g. of 2,4-D *per diem* for 21 days with no demonstrable ill-effects. A clinical study was made in Denmark by NIELSEN *et al.* (1965) on a 23-year old man who had committed suicide by apparently drinking 125 ml. of 50 percent w/v 2,4-D dimethylamine salt. The total weight of 2,4-D in his body was calculated as being not less than six g. (the equivalent of 80 mg./kg.), about 10 percent of the total weight of active material ingested. The principal damage appeared to be to nerve tissues and the central nervous system.

VALLET (1965) gives acutely toxic oral doses of 2,4-D to man of 50 to 500 mg./kg. body weight, of 2,4,5-T of 500 to 1,000 mg./kg., and of MCPA of 50 mg./kg. No indication is given of the source of these figures which are probably extrapolated from data on laboratory animals. FREIREICH *et al.* (1966) have shown that a number of drugs, on a mg./kg. basis, are more toxic in man than in the mouse, when used as a laboratory test animal, by a factor of 10 to 15. There are clearly dangers therefore in assuming that a toxic dose to man is the same as the toxic dose to a laboratory animal multiplied by some factor based on the mean differences in weight. In fact these latter authors show for a number of anticancer agents that a very much more accurate assessment of the ratio of animal to human toxicity can be obtained on a mg./m.^{2} of body surface area, than on a mg./kg. basis. For this reason VALLET's (1965) figures may be suspect, particularly in respect of the range of 50 to 500 mg./kg. for 2,4-D, although the lower figure would be of the correct order of magnitude when compared to the residue of 80 mg./kg. found by NIELSEN (1965) in his suicide case. Nevertheless, this figure represented the amount actually found and it is likely that a greater proportion of the total 2,4-D ingested was necessary to produce death. In this context it is noteworthy that the acute oral LD_{50}'s to rats of the most common organochlorine insecticides range between 10 and 135 mg./kg. (MARTIN 1963).

EDWARDS and RUPPER (1953) have discussed the hazard to operators from inhalation of sprays or aerosols during application of herbicides with particular reference to methods of protection. MONARCA and DI VITO (1961) have described a clinical study of an acute case of accidental poisoning of a man in Italy. In this instance a farmer became ill after applying a 40 percent aqueous solution of 2,4-D by handpump

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against the wind. He was admitted to hospital, suffered a relapse after 18 days, and recovered sufficiently to be discharged after 40 days. Initial symptoms of muscular weakness, vomiting, perspiring freely, and oliguria were noted in the field whilst a diagnosis of bradycardia, respiratory difficulties, and urinary abnormalities was made after admission to hospital. However the authors report that the case was exceptional. FETISOV (1966) has reported similar field symptoms in Russian workers engaged in field applications of 2,4-D. This author concluded that a range of formulations of 2,4-D was "highly toxic to animals in different ways of introduction." Whilst reports of minor discomfort following exposure to auxin sprays during field application are rarely reported in scientific literature, there is no doubt that a proportion of workers so exposed do suffer a degree of transitory discomfort. Whether this is of any significance as a long-term toxic hazard has not been determined for man.

From the very nature of their use it is unlikely that auxin herbicides will appear as significant residues in food crops. WILLIAMS (1964) was unable to detect any residues of auxin herbicides in a number of total diet samples down to the limit of sensitivity (0.01 p.p.m.) of his analytical techniques. DUGGAN and WEATHERWAX (1967) calculated pesticide chemical residues in 'total diet' samples collected on 46 days in 25 American cities during a 699 day period from June 1964 to April 1966. Each sample represented the total amount of food and drink consumed by one person over a two-week period. The total samples represented in all a food and drink supply sufficient for 644 days. Herbicide chemicals were found infrequently and averaged about 0.01 mg./day of which one third was 2,4-D, and half was MCPA and pentachlorophenol (PCP) combined. 2,4-D was found in oils and fats (0.001 mg. in 1964/65) and sugars and sugar products (0.004 mg. in 1964/65, 0.002 mg. in 1965/66), whilst MCPA was found in grain and cereals (0.002 mg. in 1964/65), in dairy products (0.003 mg. in 1965/66), and in leafy vegetables (0.001 mg. in 1965/66). These amounts are substantially below the limits set for acceptable daily intake by the World Health Organization and United Nations committees. It seems probable, therefore, that toxic hazards from auxin herbicide residues in food are very small. FAUST (1964), in a survey of water pollution hazards to man from organic pesticides, came to the conclusion that there did not appear to be danger to health at the present time from the background concentration of pesticides believed to be in ground and surface water. However, 2,4-D could persist in lake water and bottom mud for long periods under certain environmental conditions. Work in Russia, quoted by FAUST, suggested that the threshold taste and odour concentrations auxin compounds, especially of phenolic derivatives such as 2,4-D, that would prove unacceptable to the consumer were very considerably below the threshold concentrations for toxic effects. A particular risk might be supposed to lie in contaminated milk

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drawn from cows feeding in treated pasture, but no residues of either 2,4-DB or 2,4-D were found in the milk of cows that had been fed these compounds (GUTENMANN *et al.* 1963a and 1963b). The beasts were fed 5.0 p.p.m. (based on a daily ration of 50 pounds) for a number of days; the limit of detection for 2,4-DB was 0.2 p.p.m. and for 2,4-D 0.1 p.p.m. Whereas milk might be a source of contamination these results indicate that there is no apparent hazard.

III. Toxicity and hazards to domestic and laboratory animals

a) Acute and chronic toxicity

The toxicity of agricultural chemicals to land fauna is normally quoted in terms of the dose that kills 50 percent of a population of test animals (LD_{50}). Whilst this figure gives a useful indication of the comparative toxicities of different compounds to a given test species, the figures obtained in different tests may be influenced by a number of factors. Thus the age and sex of the test animal, method of dosing, and general conditions of the test may have an important bearing on the susceptibility of the animal to the compound being studied. The formulation of the active compound has a considerable influence: for instance 2,4-D acid has an LD_{50} to rats of 375 mg./kg. but the sodium salt has an LD_{50} of 805 mg./kg., the propylene glycol butyl ether ester of 570 mg./kg., and the iso-propyl ester of 700 mg./kg. (ROWE and HYMAS 1954). It should be noted, however, that BJÖRKLUND and ERNE (1966) do not regard these differences as being appreciable. In addition, the LD_{50} for different test species may vary quite widely: for example, 2,4-D acid has an LD_{50} of 375 mg./kg. for rats, of 100 mg./kg. for dogs, 469 mg./kg. for guinea pigs, and 541 mg./kg. for chicks (ROWE and HYMAS 1954).

Bearing in mind these reservations, the acute oral LD_{50} to rats of the acid formulations of the compounds under consideration are: 2,4,5-T 300 mg./kg., 2,4-D 400 mg./kg., MCPB 680 mg./kg., MCPA 700 mg./kg., 2,4-DB 700 mg./kg., 2,3,6-TBA 705 mg./kg., and mecoprop 930 mg./kg. (WOODFORD and EVANS 1965). Thus, 2,4-D and 2,4,5-T fall in the moderately toxic and the other five compounds in the slightly toxic class according to the widely accepted classification of toxicity of chemicals quoted by FRAZER (1963). In a comparison of the relative toxicities of a number of commercial formulations of 2,4-D and 2,4,5-T, ROWE and HYMAS (1954) concluded that the 'inert' constituents of the formulations did not add to the toxicity of the active compound, nor did they exact any potentiating effect. Similar conclusions were reached by DALGAARD-MIKKELSEN and POULSEN (1962) on the basis of this and other work. However, it is possible for surfactants and other additives to enhance the toxicity of active compounds. Whilst there is some evidence with insecticides that mixing

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of compounds leads to synergistic effects on the acute toxicity of the constituent compounds (KEPLINGER and DEICHMANN 1967), effects of this sort have not been reported for herbicide mixtures in common use. However, the possibility of their occurring should not be overlooked, nor, also, the possibility of antagonism where the effect of one compound upon another in a mixture is to depress its biological activity.

Descriptions of the clinical symptoms of poisoning by MCPA, 2,4-D, and 2,4,5-T of a range of experimental animals have been summarized by ROWE and HYMAS (1954) and more recently reviewed with comments by DALGAARD-MIKKELSEN and POULSEN (1962). BJORKLUND and ERNE (1966) have subsequently further reviewed and reported on their own results on the effects of feeding 2,4-D to calves, pigs, rats, and chickens. A range of symptoms was produced that was generally similar to those described by the previous authors. ERNE (1966a and b) studied the distribution and elimination of 2,4-D and 2,4,5-T in these animals. Amine and alkali salts of both compounds were readily absorbed and completely distributed in the body, but 2,4-D ester was incompletely absorbed and reached only a low level in the plasma and tissues. The highest tissue levels of the two compounds were found in liver, kidney, spleen, and lungs and the levels found in these organs sometimes exceeded the level found in the plasma. In blood cells some 10 to 20 percent of the plasma level was found. Penetration of 2,4-D into placental tissue of pigs was recorded but there was little or no evidence of penetration into adipose tissue or the central nervous system. Elimination of the compounds was rapid, the plasma half-life being about three hours in rats, eight in calves and chickens, and 12 in pigs. The tissue half-life values ranged between five and 30 hours. No retention of the compounds was noted in the tissues. There was no accumulation after repeated dosing and in pigs there was an increase in the rate of elimination after repeated administration. In all species the main excretory route was *via* the kidneys. KHANNA and FANG (1966) traced the metabolism of C^{14}-labelled 2,4-D in rats dosed at rates from one to 100 mg./animal. Radioactivity was found in all the organs studied together with some accumulation as early as one hour after dosing. At the one mg. dose rate a concentration peak of radioactivity was demonstrated after six to eight hours but decreased thereafter and was non-detectable by 24 hours. At the 80 mg. dose the peak occurred at eight hours and persisted for 17 hours. Extracts of the tissues were shown to contain mainly unchanged 2,4-D residues. No radioactivity was found in the expired carbon dioxide, but elimination in urine and faeces was dose dependent. At the one to 10 mg. doses 93 to 96 percent of the ingested 2,4-D was excreted unchanged in the urine in the first 24 hours. At the 20 to 100 mg. doses greater amounts of 2,4-D were found in the second 24 hour period after dosing, with a linear decrease in percentage recovery with increase in dose. In experiments with cattle GUTENMANN *et al.* (1963a and b) were un-

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able to detect any residues of 2,4-DB or 2,4-D in milk or faeces of cows fed five p.p.m. of either compound in a 50 pound daily ration. In these experiments there was no evidence of beta-oxidation of 2,4-DB to 2,4-D. Disappearance of 2,4-D was thought to occur as a result of dilution in the rumen, some absorption on the gut wall, and by decomposition. In subsequent experiments Bache *et al.* (1964) and St John *et al.* (1964) studied the fate of MCPA, MCPB, 2,4,5-T, and a number of other herbicides in cattle. All the MCPA fed to a single steer (113.5 mg. single dose based on five p.p.m. of a 50 pound daily ration) was accounted for in its urine over the four days after administration. No MCPB was found in the urine of MCPB-dosed cows (total dose of 56.75 mg. and 113.5 mg. based on 2.5 and 5.0 p.p.m. of a 50 pound daily ration) on the first day after administration, but subsequent recoveries in their urine showed that 7.2 to 9.2 percent of the MCPB was converted to MCPA. Analysis of MCPB itself, however, was said to be unsatisfactory. It is of interest that a similar conversion of 2.4-DB to 2,4-D can be performed by bluegill sunfish (*Lepomis machrochirus*) (Gutenmann and Lisk 1965). Urine and milk samples were also analysed from cows fed a total of 454 mg. of 2,4,5-T over a period of four days. It was shown that the compound was excreted exclusively in the urine over a period of six days, a total of 430.7 mg. being recovered. Clarke *et al.* (1964) investigated the fate of C^{14}-labelled 2,4-D in sheep fed a dose of four mg. of 2,4-D/kg. calculated as the minimum daily dose likely to be ingested from grazing treated pasture. The level of radioactivity in the blood rose to a peak 1.5 hours after dosing but fell to the normal background level in 24 hours. All the material was excreted within 72 hours, with over 98 percent in the urine and only 1.5 percent in the faeces. Balayannis *et al.* (1965) dosed a rabbit and six mice with 2,3,6-TBA and showed that a proportion of the compound was recovered after passing through the animals. These authors discussed the significance of biologically active residues of auxin compounds in animal excreta that might become incorporated in manure or straw. It was shown that 2,3,6-TBA residues in particular could remain active for a period of months and affect susceptible crops to which the contaminated manure was applied. The same hazard does not normally exist with the phenoxyacetic acid derivatives, where the compounds are broken down and become biologically inactive in a relatively short period of time. However, Lisk (1966) has pointed out that the excretion of 2,4-D in the urine of cows does present the admittedly remote possibility of active 2,4-D being transferred from a treated pasture to a susceptible crop.

Mitchell (in Kephart 1945), Dalgaard-Mikkelsen *et al.* (1959), and Goldstein and Long (1960) all reported that there were no apparent ill-effects in cattle, sheep, or horses from grazing pasture sprayed at herbicidal or two times herbicidal rates of 2,4-D or MCPA. Grigsby and Farwell (1950) (in Springer 1957) reported that there

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was no significant difference in the amount of feeding of horses, cows, sheep, and pigs in untreated plots or plots sprayed with the sodium salt or the isopropyl ester of 2,4-D, or the isopropyl ester of 2,4,5-T. However, there did appear to be less feeding in plots sprayed with the alkanolamine salt of 2,4-D. There was no effect on milk production of cows feeding on sprayed vegetation. GOLDSTEIN and LONG (1960) found no ill-effects on two cattle from adding 0.25 pints of a 1.5 percent w/v 2,4-D/2,4,5-T mixture to every five gallons of their drinking water for 41 days. These authors also reported spraying the skins of a calf, of a cow, of sheep, and of pigs with doses ranging from 0.002 to 0.008 pounds of 2,4-D or 2,4-D/2,4,5-T mixture, with no ill-effects. These dose rates would be of the order of those that might occur in an instance of spray drift.

DOBSON (1954) sprayed MCPA, 2,4-D, or 2,4,5-T on grassed chicken runs daily for 14 days at normal and ten times normal dose rates. 2,4,5-T significantly reduced egg production and the weight of the birds; MCPA and 2,4-D also affected egg production, mainly in the second week of spraying or during the week after spraying had stopped. In all instances there was no effect on the fertility of the eggs and all the progeny reared well, although the dose rates and frequency of application in this trial were much more severe than are likely to be found in practice. ERNE (1966) showed that some of the 2,4-D fed to hens could be excreted in their eggs. DUNACHIE and FLETCHER (1967) injected hen's eggs with MCPA, 2,4-D, MCPB, 2,4-DB, mecoprop, and 2,3,6-TBA amongst a range of other herbicides. Dose rates were 10, 100, and 200 p.p.m. equivalent to 0.5, 5, and 10 mg./egg. The percentage hatch was recorded. At the lowest dose there was 100 percent hatch from the MCPA- and MCPB-treated eggs; 90 percent from the mecoprop- and TBA-treated eggs, and 80 percent from the 2,4-D-treated eggs. At the highest dose there was 80 percent hatch from mecoprop, 50 percent from 2,4-D and TBA, and 20 percent from MCPA and MCPB. None of the chicks that hatched was deformed although some feather blanching was noted from the MCPA, MCPB, 2,4-DB, and meco[illegible] treatments. ROBERTS and ROGERS (1957) reported on various feeding experiments on turkeys with alfalfa sprayed with a low volatile ester of 2,4,5-T at herbicidal rates. No deleterious effects were noted. Calculations were quoted to show that for a one kg. chicken to acquire a lethal dose of 2,4-D from an application rate of one pound/acre, the bird would have to consume in two days all the 2,4-D applied to the vegetation over an area of 72 square feet. Similar calculations by ROWE and HYMAS (1954) for cattle suggested that for a 770 pound animal to acquire an acutely toxic dose of 2,4-D of approximately 1,000 mg./kg., it would have to graze completely and ingest all the 2,4-D from an area of 0.17 acre (one pound/acre = 10.41 mg./ft.^{2} = 93.7 mg./yard^2 = 112.1 mg./m.^{2}).

Accounts are given of direct oral dosing or dermal applications of

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* 1 lb/A = 10.41 mg/ft^2

1000 mg/kg in a 350 kg animal = 350,000 mg

≈ 35,000 sq ft = 0.8 acre

M. Kang 11/4/69

WWW 1546800

auxin herbicides to a variety of domestic animals by KEPHART (1945) (cow), ROWE and HYMAS (1954) (laboratory animals and cattle), DALGAARD-MIKKELSEN *et al.* (1959) (heifers), PALMER (1963) (cattle), PALMER and RADELEFF (1964) (sheep and cattle), CLARKE *et al.* (1964) (sheep), and STRACH and BOHOSIEWICZ (1964) (pigs). PALMER (1963) gave daily oral doses of 2,4-D alkanolamine salt to steers for five days in every seven. He recorded signs of poisoning in animals dosed at 250 mg./kg. after 15 administrations as opposed to 86 administrations of 100 mg./kg.; at 50 mg./kg. no ill-effects were recorded over a period of 112 administrations. From these results he concluded that although animals could probably ingest enough 2,4-D from concentrated solutions at any one time to produce illness or death, the chronic toxicity of the compound was sufficiently low to make it unlikely that an animal would pick up enough of it over a period of time to cause any serious ill-effects. Further work by PALMER and RADELEFF (1964) using single animals gave the following results:

1. Sheep tolerated 481 daily doses of 100 mg./kg. of the alkanolamine salt or propylene glycol butyl ether ester of 2,4-D.
2. Cattle suffered from chronic tympanites after 88 daily doses of 100 mg./kg. of the alkanolamine salt of 2,4-D. One animal died after 34 daily doses of 200 mg./kg.
3. Sheep tolerated 481 daily doses of 100 mg./kg. of the triethylamine salt of 2,4,5-T but succumbed to 369 doses of 100 mg./kg. of the propylene glycol butyl ether ester.
4. Sheep were killed by 383 daily doses of 100 mg./kg. of MCPA amine.

The toxicity of 2,3,6-TBA in these trials was said to be slightly greater than that of 2,4-D or 2,4,5-T. STRACH and BOHOSIEWICZ (1964) reported that no abnormal behaviour in pigs had been noted following 40 daily doses of 15 to 100 mg./kg. of 2,4-D, nor from single doses of 2,4-D of 200 to 800 mg./kg.

In short term trials by BJÖRKLUND and ERNE (1966), calves and pigs showed definite though reversible symptoms of poisoning after single doses of 2,4-D of 200 and 100 mg./kg., respectively. Rats and fowls did not show any sign of distress after single doses of 100 and 300 mg./kg., respectively, and fowls tolerated daily doses of 300 mg./kg. daily in their feed for several weeks without visible effects. Repeated daily doses of 50 mg./kg., however, led to toxic symptoms in some pigs. In longer term studies (ERNE 1966a), five young pigs were fed 500 p.p.m. of 2,4-D for up to 12 months but, although various toxic effects were noted and their growth rate was affected, none of the animals died. When 2,4-D was fed to a sow throughout gestation and for a further six weeks, 10 of the 15 underdeveloped and apathetic piglets she produced died within 24 hours and the mother subsequently had to be slaughtered because of abnormalities that developed in her spine. Heavy dosing of pregnant rats, however, with 1000 p.p.m. of

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DOW 1546801

2,4-D in their drinking water over 10 months and of their off-spring for up to two years, whilst leading to retarded growth and increased mortality, did not produce unequivocal signs of toxicity. Continued administration of 500 p.p.m. of 2,4-D in feed or 1,000 p.p.m. in the drinking water of fowls led to reduced egg production and kidney abnormalities. These results led the authors to conclude that the chronic toxicity of 2,4-D to the species studied was moderate. They were, however, concerned about the mortality of new-born piglets, with evidence of movement of 2,4-D through the placental tissues, and the reduced egg production in fowls which they thought might indicate a possible interference with reproductive processes.

In general the findings of other workers support these conclusions on acute and chronic toxicity. In all the work quoted the amounts administered to the test animals for effect, have been well in excess of the amounts they might be expected to pick up from a treated pasture, or in feed derived from crops that had at some time been treated with auxin herbicides at normal dose rates.

b) Indirect toxicity

Indirect effects of herbicides on grazing animals have been associated with increased toxicity of toxic plants, increased palatability of normally non-palatable toxic plants (e.g., ragwort, *Senecio jacobaea*), and induced toxicity in normally nontoxic plants (e.g., temporary increases in nitrate content) (WILLARD 1950). However, FERTIG (1953) claimed that, up to 1953 in America, in all cases where poisoning of livestock from herbicides had been reported, the effects noted could be attributed to some other cause.

Examples have been given by WILLARD (1950) of cattle eating wild cherry (*Prunus serotina*), of pigs eating Cocklebur (*Xanthium* sp.), and of lambs eating thistles after herbicidal treatment with auxins. Instances have been reported of ragwort becoming 'sweeter' from two or three days after application and being preferentially grazed by cattle for a short period. GRIGSBY and BALL (1952) and LYNN and BARRONS (1952) investigated the hydrocyanic acid (HCN) content of the leaves of wild cherry from untreated trees and trees treated with 2,4-D and 2,4,5-T. Their conclusions were that the foliage was no more toxic to cattle after treatment and that there might even be less HCN in the leaves of the treated trees than in those of the untreated ones. BUCK *et al.* (1961) fed the alkaloid-containing plants *Delphinium barbeyi* (tall larkspur) and *Helenium hoopsii* (sneeze weed), after treatment with 2,4-D ester or 2,4,5-T ester, to calves and ewes. No increased toxicity of the plants attributable to application of the herbicides was noted. WILLIAMS and CRONIN (1963) analysed *D. barbeyi*, treated with 2,4,5-T amine at various growth stages, and showed that the alkaloid content of the plants was increased for several

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weeks after treatment at the vegetative and early bud stages. It was noted, however, that the bitter taste of the alkaloids might make the treated plants even less palatable to animals than untreated ones.

SWANSON and SHAW (1954) showed that 2,4-D affected the HCN content of Sudan grass (*Sorghum vulgare* ssp. *sudanense*). Initially there was a decrease in the content of HCN but four days after treatment there was an increase over the controls which was maintained for a further 12 days. Similar effects were shown to occur with the nitrate content of leaves. BUCK *et al.* thought that there might be a relationship between HCN and nitrate metabolism in Sudan grass, an increase in one leading to a decrease in the other.

The clinical aspects of nitrate poisoning in stock, conditions under which nitrates are likely to accumulate in the leaves of certain plants, and lists of these plants have been reported by BRADLEY *et al.* (1940), DAVIDSON *et al.* (1941), GILBERT *et al.* (1946), CASE (1957), and SUND *et al.* (1960). The toxic effects of nitrate are caused by a reduction of nitrate to nitrite and the conversion by nitrite of haemoglobin in the blood to methoglobin: the animal dies from asphyxia. Intravenous injection of methylene blue in doses of two g./500 pounds of body weight gives immediate relief. Nitrate in plants is generally present in the form of potassium nitrate and increases in nitrate content have been associated with drought conditions and high soil nitrogen (GILBERT *et al.* 1946, CASE 1957). SUND *et al.* (1960) noted a high nitrate content in *Urtica* spp. and *Rubus* spp. after heavy rains, followed by preferential grazing of these and other weed species by cattle. A number of abortions in these cattle was correlated with occurrence of high nitrate rather than grazing of the weed species *per se*. Recent increases in vitamin A deficiency in North American ruminants has been associated with ingestion of nitrates occurring in herbicide-treated plants by PHILLIPS (1964).

The accumulation of nitrates in the leaves of sugar beet is well known (*e.g.*, SAVAGE 1949). Increased levels of nitrate in the leaves of this crop as a result of herbicide application have been reported by WILLARD (1950), STAHLER and WHITEHEAD (1950), and WHITEHEAD *et al.* (1956). Isolated incidents have been reported of nitrate poisoning of cattle in America as a result of feeding on sugar beet that had previously been sprayed. In one incident in N. Dakota, the nitrate content of sugar beet leaves after spraying was found to vary from 1.81 to 8.77 percent of the dry weight, as against 0.22 percent for untreated plants and a toxic level of 1.5 percent (STAHLER and WHITEHEAD 1950). Studies on forage crops (BERG and MCELROY 1953) and on a range of weed species (FRANK and GRIGSBY 1957) have shown which of these may contain high levels of nitrates after auxin application. Cell-free extracts of maize and cucumber from plants that had been previously sprayed with 10 and 100 p.p.m. of 2,4-D were investigated by BEEVERS and HAGEMAN (1962). The level of nitrate reductase was

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increased in maize but reduced in cucumber. Studies on the formation and breakdown of nitrates in plants (Fertic 1952, Freiberg and Clark 1952, Whitehead *et al.* 1956) have shown that 2,4-D causes more rapid increases in nitrate content than MCPA, that levels rise to a peak soon after spraying and subsequently decrease with time, and that increases in light intensity hasten decreases in nitrate content.

It is clear from these reports that nitrate poisoning in stock does occur from time to time and that it is possible for the hazard to be increased by application of auxin herbicides to nitrate-accumulating plants.

IV. Bees and other insects

Herbicides affect bees (*Apis mellifera*) and other insects if they kill the plants on which the insects feed. In addition, Wahlin (1950) has reported that 2,4-D and MCPA were toxic to bees, not only from visiting the flowers but also as a result of drinking contaminated water trapped on treated plants. Antoine (1966) reported that MCPA paralysed and killed bees which ingested doses that corresponded to those recommended for weed control. Other workers have reported effects on bees after application of auxin herbicides to plants in flower but not at other times (Haragsimova 1962, Palmer-Jones 1964). Palmer-Jones (1964) and Antoine (1966) have suggested that 2,4-D might have some effect on nectar which made it toxic to bees. King (1960a) has shown that radioactive 2,4-D can be translocated to the nectar of *Poinsettia* and red clover plants and may be detectable there for two to three days after treatment. Feeding trials of auxin herbicides to bees have been reported by Glynne Jones and Connell (1954), Palmer-Jones (1960), King (1960b), and Byrdy (1962). Palmer-Jones (1964) found no effect on bees that had been directly dusted with 2,4-D or when they were made to crawl through 2,4-D dust in order to enter the hive. Glynne Jones and Connell (1954) classed 2,4-D and MCPA as stomach/contact poisons of low toxicity to bees, with LD_{50} values of 0.105 mg. compared to insecticides in the range 0.00004 to 0.002 mg. Byrdy (1962), on the other hand, reported total mortality of bees within four days of feeding 30 μg. of 2,4-D and 10 percent mortality within three days rising to 20 percent in five days of feeding 20 μg. Johansen (1959) reported that 2,4-D and related compounds were not toxic to bees, except when formulated as the alkanolamine salt or the isopropyl ester. Haragsimova (1962) found that a 30 percent calcium/potassium salt formulation of MCPA was toxic to bees and caused mortality up to 13 days after ingestion. However, part at least of the loss was attributed to cresol impurities, which led to loss of smell amongst the bees with consequent loss of sense of direction.

Occasional observations on other insects have been reported.

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MELLANBY *et al.* (1959) observed that a spray of mecoprop on oats at the time of a heavy frit fly attack had no effect on the fly (*Oscinella frit*). MAXWELL and HARWOOD (1960) treated broad bean (*Vicia faba*) plants with sublethal doses of 2,4-D and recorded a marked increase in the reproduction of the pea aphid (*Macrosiphum pisi*) feeding on them. The longevity of adult aphids was unaffected. ROBINSON (1959) also recorded increased fecundity in another pea aphid (*Acyrthosiphon pisum*) after caging on broad bean plants treated with 2,4-D. ADAMS (1960) and ADAMS and DREW (1965) showed that the application of 2,4-D amine could enhance aphid infestation in New Brunswick grain fields, probably as a result of depressing the activities of coccinellid beetles predating on the aphids. In laboratory experiments with coccinellid larvae treated with 2,4-D amine, there was a fourfold increase in mortality and an increase in time to pupation. There was little mortality amongst the adult beetles, which usually recovered after a few hours inactivity. ISHII and HIRANO (1963) concluded that increases in the growth rate of the larvae of the rice stem borer (*Chilo suppressalis*) feeding on 2,4-D treated rice plants, was a consequence of increased nitrogen content of the plants rather than a direct effect of the chemical itself.

In general, it appears that there is a real hazard to bees—and possibly other nectar feeding insects—from applications of auxin herbicides to plants in flower. Otherwise there would seem to be little hazard to insects from direct toxicity of the compounds at normal herbicidal rates of application. However, the work of ADAMS and DREW (1965) does suggest that some insects may be more susceptible at particular stages of their life cycle than at others.

V. Soil animals

Amongst soil arthropods, DAVIS (1965) was unable to show any differences in populations of *Acari or Collembola* species between untreated plots and plots that had been sprayed with MCPA in 10 out of 13 years. DAVIS (1965) was unable to find any reports of significant effects on soil animals in other experiments of a similar nature. BOLLEN (1961) concluded that auxin herbicides, based on phenoxyacetic and propionic acid, were the most susceptible to breakdown by microorganisms of the many pesticides applied to the soil. The importance of soil microorganisms in the breakdown of these herbicides is well known from the work of AUDUS (1964) and others. WEBSTER (1967) has briefly reviewed the literature on the influence of plant growth-regulator auxin herbicides on the host/parasite relationships of nematodes, in which 2,4-D has been shown to increase nematode reproduction in plant callus cultures. In addition, plant cell hypertrophy and proliferation, which is a common effect of 2,4-D in many plants, provides highly suitable conditions for development of nematodes. In

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this way susceptibility of a normally nematode-resistant variety of oats could be induced, although there did not appear to be any greater susceptibility of a non-resistant variety.

VI. Fish and aquatic organisms

Under field conditions the toxicity of a pesticide in water is affected by a number of factors in addition to those that affect its performance on land. Thus acidity, hardness of the water, and the sorbent qualities of suspended organic matter in the water may directly effect the toxicity. The trophic nature of the ecosystem, the oxygen status of the water in respect of both producers and demand, and the amount of movement of water both within the system and in terms of flow will affect the concentration of the chemical, its persistence, and its possible toxic side effects. Because of these, and many other interacting factors, the toxicity of a given formulation of a given chemical compound to an individual species will vary under field conditions depending upon the nature of the water body and the immediate environment. For this reason toxicities to fish and aquatic organisms are usually estimated in terms of median tolerance limit for exposure to a given concentration of the pesticide, for a given length of time (TLmx).

In addition to direct or indirect toxicity, the effects on aquatic organisms of the removal of the substrate that gives them food and shelter must also be considered. For instance, in one of the Tennessee Valley Authority's reservoirs two applications of 2,4-D controlled considerable acreages of Eurasian water milfoil (*Myriophyllum spicatum*). The eradication of the plant eliminated the substrate that might have been colonized by large populations of epiphytic insects such as the larvae of midges, mayflies, and dragonflies (SMITH and ISOM 1967). Although under some conditions this might have a very serious effect, it has been shown by WAY *et al.* (1968) in an ecological investigation of the use of paraquat in lakes, that although individuals and populations of animals may be severely affected in the weeks after application of a herbicide, the species affected may be present again in the season after treatment. It has also to be recognized that very heavy infestations of submerged or floating aquatic plants may interfere with the passage of nutrients and considerably reduce the temperature and dissolved oxygen values of the water (FISH 1966). Thus, any possible hazards from the use of a herbicide may be outweighed by the advantages gained from the removal of the vegetation.

The danger of significant amounts of 2,4-D appearing in ground water or streams as a result of local applications seems to be slight. ALDHOUS (1967) measured residues of 2,4-D in drainage channel water after aerial spraying of a Scottish forest at four pounds/acre active ingredient in 12 gallons of water. Up to two p.p.m. of 2,4-D was measured in the seven days after application but none was detectable

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by 28 days. BROWN and NISHIOKA (1967) analysed samples of a water-suspended sediment mixture from 11 streams in the western United States at monthly intervals. Amongst the analyses made were gas chromatograph determinations for 2,4-D and 2,4,5-T. Neither compound was found in any of the samples. The absence of the compounds was attributed to some extent to their susceptibility to degradation.

Reviews of toxicity hazards to fish of a range of pesticides, including auxin herbicides, have been made by BAUER (1961), BANDT *et al.* (1962), and COPE (1965 and 1966). COPE (1966) noted that variations in formulation gave rise to greater differences in toxicity than the differences in toxicity between the basic compounds. Ester formulations were often more toxic than amine or metallic salt formulations. Similar observations were made by LHOSTE (1959) who reviewed effects on a number of crustaceans, aquatic insects, and molluscs.

Trout (*Salmo trutta*) are normally regarded as being amongst the most sensitive fish to water pollution. ALABASTER (1958) has given median tolerance limits for 24 and 48 hour (TLm_{24} and TLm_{48}) exposures of trout to 2,4-D or 2,4,5-T, or to mixtures of these two compounds, of 9.5 to 250 p.p.m., depending on formulation, compared to 1,150 to 2,000 p.p.m. for sodium chlorate or 0.005 p.p.m. for phenyl mercuric acetate. HOLDEN (1964) devised a formula for comparing the likely toxic hazards to trout from a number of pesticides [1] applied at agricultural rates. The following comparative estimates of hazard were given: aldrin = 70, PCP = 7, MCPA = 1.5, 2,4-D = 1, 2,4,5-T = 0.5, paraquat = 1/12, simazine = 1/27, diquat = 1/40, dalapon = 1/46, TCA = 1/120, and aminotriazole = 1/150. It should be emphasized that these figures refer to agricultural rates of application: hazards from the concentrates might be different.

Perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) are unlikely to be affected by MCPA, 2,4-D, or 2,4,5-T (BANDT 1957) at rates of application used for aquatic weed control, although a commercial mixture of 2,4-D and 2,4,5-T was more hazardous. In later trials BANDT *et al.* (1962) found threshold values for toxicity to perch and roach of MCPA of 200 to 215 mg./litre, of 2,4-D of 75 mg./litre, of 2,4,5-T of 55 to 60 mg./litre, and of 2,4-D + 2,4,5-T mixtures of 5 to 12 mg./litre. These results show rather greater toxicities of 2,4-D and 2,4,5-T compared to MCPA than were shown in HOLDEN's (1964) calculations (see above). The much enhanced toxicity of the 2,4-D + 2,4,5-T mixtures may have been an effect of the particular formulations used, or

[1] Aldrin = 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*exo*-1,4-*endo*-5,8-dimethanonaphthalene; PCP = pentachlorophenol; paraquat = 1,1'-dimethyl-4,4'-bipyridylium dichloride; simazine = 2-chloro-4,6-bisethylamino-1,3,5-triazine; diquat = 9,10-dihydro-8a,10a-diazonaiaphenanthrene dibromide; dalapon = sodium 2,2-dichloropropionate; TCA = trichloroacetic acid; and aminotriazole = 3-amino-1,2,4-triazole.

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could demonstrate a synergistic effect of one compound on the other. DAVIS and HARDCASTLE (1959) established median tolerance limits over a 24 hour period (TLm_{24}) for bluegill sunfish (*Lepomis macrochirus*) to a number of herbicides. Values obtained when the compounds were added to relatively pure water were 2,4-D = 39 p.p.m., MCPA = 20 p.p.m., 2,4-DB = 20 p.p.m., and 2,3,6-TBA = 1,800 p.p.m. COPE (1966) noted delays in spawning of bluegill sunfish of up to two weeks after treatment of water with the propylene glycol butyl ether ester of 2,4-D at five and 10 p.p.m. However, no other effects were noted on reproduction or on survival of fry. In pond experiments, death of some fish as a result of 2,4-D treatment led to increased size in the survivors, probably as a result of the greater food supply available to the individual fish. In further trials with bluegill sunfish, HUGHES and DAVIS (1963) and DAVIS and HUGHES (1963) reported on effects of different formulations of 2,4-D and other auxins. Their tests showed 2,4-D and 2,4,5-T esters to have TLm_{24} ranging from 1.8 to 10 p.p.m. depending on the ester used. Dimethylamine salts of 2,4-D and 2,4,5-T had TLm_{24} of 162 to 542 p.p.m. and 144 p.p.m., respectively, compared to the alkyl amine salt of MCPA of 163.5 p.p.m. and of 2,4-D acid of 8.0 p.p.m. This work (which is referred to in COPE 1966, see above) shows the wide differences in toxicity that can occur in different formulations and the care which must therefore be taken in assessing the toxicity of an individual product before recommending it for use as an aquatic herbicide.

In addition to work on fish, WALKER (1962) has reported effects on a variety of bottom-feeding fish food organisms following application of 2,4-D to plastic enclosures at 1.0 to 4.0 p.p.m. LIROSTE (1959) has reported that ester formulations of 2,4-D or mixtures of 2,4-D and 2,4,5-T affected crustaceans, aquatic insects, and molluscs in the range of 0.1 to 3.3 p.p.m. In their investigations into the effect of the use of 2,4-D at rates from 40 to 100 pounds/acre in Tennessee Valley Authority reservoirs, SMITH and ISOM (1967) found no measurable toxic effect on benthic fauna or significant changes in mean numbers of burrowing mayflies (*Hexagenia*) before or after treatment. There were some deaths of caged fish but no dead or distressed free fish were found. Observations of free living fish indicated that they appeared to move out of the treated area, whilst analysis of a number of fish for 2,4-D residues gave no measurable results at the limit (0.14 p.p.m.) of detection. However, analysis for residues in mussels (mainly *Elliptio crassidens*) indicated that they concentrated the chemical from the surrounding water although no mortality was recorded. Analysis of mud samples showed that significant amounts of 2,4-D (up to 58.8 p.p.m. butoxyethanol ester) were present in isolated sediment samples up to 10 months after treatment. These fairly high levels in the environment of a sedentary animal such as the mussel might explain the residues found in these animals. RAWLS (1965) has reviewed literature

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on the toxicity of herbicides to estuarine fauna, principally crustacea and mollusca, and described experiments from his own laboratory. A variety of formulations of 2,4-D affected several species of animals at concentrations of one to 10 p.p.m. Larvae of oysters (*Crassostrea virginica*) were killed over a period of time by one p.p.m. of butoxyethanol ester and dimethyl salt of 2,4-D, and though oyster eggs were less susceptible, their development could be affected by 2,4-D butoxyethanol ester at 10 p.p.m. These effects might give some cause for alarm when rates of application of 20 to 30 pounds/acre are required for weed control in estuaries. Doses of this sort would give 7.34 to 11.02 p.p.m. if evenly distributed throughout one acre of water one foot deep. Because of tidal fluxes and uneven distribution only very local concentrations at toxic or near toxic amounts may occur in practice.

The results of these various investigations suggest that at herbicidal rates of application of auxins the hazards from acute or chronic toxicities to aquatic organisms are low. Nevertheless in some instances the dose rates required for effective herbicidal action, for example in estuaries or where the chemical is likely to be rapidly dispersed, may give rise to local and perhaps short term concentrations not far removed from those required for toxic effects on some organisms at susceptible stages of their life history.

VII. Wildlife in general

Hazards to wildlife from auxin herbicides have been reviewed by Rudd and Genelly (1956), Springer (1957), and Mellanby (1967). Whilst with any material having biological activity a risk of acute or chronic toxicity is always present, authenticated incidents of widescale poisoning of wild animals by these herbicides have not been reported. However, there is the further hazard to all forms of wildlife from herbicides of altering the habitat. Whilst herbicidal applications of the compounds to agricultural land undoubtedly modify the habitat there for wildlife, it is unlikely that individual instances of spray drift of the compounds at sublethal doses, although undesirable, have any really significant effect on habitat of adjoining non-cultivated land. This is not to say that some food plants for animals are not regularly killed in hedgerows and other field margins from spray drift, nor that in isolated instances of gross mishandling more severe effects do not occur over larger areas. It is probably true, although there is little or no scientific evidence, that the continued use of herbicides in the U.K. is gradually leading to the impoverishment of the flora of the field margins, and consequently to the gradual impoverishment of agricultural land as a whole as a habitat for wildlife.

The principal problem from the use of auxin herbicides in relation

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to wildlife, is not toxicological but ecological in terms of scale and intensity of use, especially on non-agricultural and industrial land. Thus the total destruction of a small area of vegetation on one occasion might be less serious for the wildlife of a region than the selective destruction of vegetation over a wide area at regular intervals. The hazard from the use of herbicides lies in the fact that they can now be used to affect vegetation over very wide areas in a short period of time, thus eliminating 'reservoirs' of wildlife and wildlife habitat that would have otherwise survived.

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Summary

Toxicities and hazards to man, domestic and laboratory animals, wildlife, bees, and other insects, soil animals and fish from MCPA, MCPB, 2,4-D, 2,4-DB, 2,4,5-T, mecoprop, and 2,3,6-TBA are discussed. In man only one authenticated instance of death by poisoning has been noted in West European and North American literature. Authenticated case histories of sublethal effects are also very rare. However, complaints of transient dizziness, sickness, and other symptoms are made from time to time by workers engaged in field applications, especially under conditions where the spray is inhaled excessively. The possibility of man acquiring toxic doses of these compounds in food, milk, or water appears to be very low.

Toxicity tests on a range of laboratory and domestic animals with a wide range of formulations of the compounds tend to show greater differences between formulations than between compounds. Amongst the domestic animals studied dogs appear to be more susceptible than the others. Acutely toxic doses of the compounds to farm animals and fowls are shown to be greater than the animals could normally ingest from treated vegetation or water following an agricultural application. Hazards to stock from the concentrated materials are emphasized, but the offensive and unpalatable nature of the undiluted chemicals would probably preclude any normal animal from ingesting a toxic dose. The danger of chronic toxicity is also shown to be very low and continuous high dosing over periods of months rather than weeks are required to produce severe symptoms of poisoning. In many animals a high proportion of the active chemical is voided in the urine within 24 to 48 hours of ingestion. Secondary hazards to animals as a result of changes in the chemical constituents of food plants may occur, for instance toxicity has been associated with temporary increases in the nitrate contents of some plants after spraying. Current recommendations for stock to be excluded from treated pasture for a period after treatment should be observed.

Hazards to wildlife exist from the use of herbicides but are also associated with other changes in agricultural practices. The effects of herbicides on habitat are serious for wildlife and should not be

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underestimated. Deaths of bees have occurred following spraying of nectar-producing plants when in flower. It is possible that similar hazards exist for other nectar-feeding insects. Where other insects have been studied, increases in population have been noted more often than decreases. With some aphids this has been associated with reduction in numbers of predators. No effects have been noted in studies of populations of soil arthropods. Increased activity by nematodes has been associated with the favourable conditions for invasion of the host plant created by auxin induced teratomas and other effects. Toxicity to fish and other aquatic organisms has been shown to depend on the formulation of the compound used. In general, toxicity to fish is low and herbicidal applications to water do not present a severe direct hazard. The dangers of secondary effects must be assessed in relation to other methods of control and the environmental characteristics of the water body to be treated. Some aquatic organisms other than fish may be directly affected at dose rates not very much in excess of those recommended for herbicidal use in water.

Résumé *

Sont discutés les toxicités et les dangers, pour l'homme, les animaux domestiques et de laboratoire, la vie sauvage, les abeilles et autres insectes, la faune du sol et les poissons, des composés suivants: MCPA; MCPB; 2.4.D.; 2.4.DB; 2.4.5.T; mecoprop et 2.3.6.TBA. Chez l'homme, un seul cas authentifié de mort par intoxication a été relevé dans la bibliographie de l'Europe occidentale et de l'Amérique du Nord. De même, les cas authentiques d'effets subléthaux sont très rares. Néanmoins, des sujets affectés à l'épandage de ces produits se plaignent, de temps à autre, particulièrement lorsque les conditions de dispersion exposent à une inhalation excessive, de vertiges passagers et d'autres symptômes et se sentent malades. L'éventualité d'absorption par l'homme de doses toxiques avec les aliments, le lait et l'eau semble très limitée.

Les épreuves de toxicité sur une grande variété d'animaux de laboratoire et d'animaux domestiques tendent à montrer que les différences sont beaucoup plus grandes en ce qui concerne les formulations qu'en ce qui concerne les matières actives. Parmi les animaux domestiques, le chien paraît être le plus sensible. Les doses susceptibles de provoquer des effets de toxicité aigüe chez les animaux de ferme et les volailles sont supérieures à celles que ces animaux pourraient ingérer à partir des plantes traitées ou de l'eau éventuellement polluée à la suite des applications en Agriculture. L'accent est mis sur les risques pour le bétail des formulations concentrées, mais la nature agressive et le mauvais goût de ces formulations rendent très impro-

* Traduit par R. Truhaut.

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bable l'ingestion d'une dose toxique par un animal normal. Les risques de toxicité chronique sont également très faibles, car des doses élevées doivent être répétées, non seulement pendant des semaines mais pendant des mois, pour pouvoir provoquer des symptômes graves d'empoisonnement. Chez beaucoup d'animaux, une forte proportion des matières actives est excrétée dans l'urine dans les 24 à 48 heures qui suivent l'ingestion. Des risques secondaires pour les animaux peuvent résulter de modifications dans les constituants chimiques des végétaux consommés; par exemple, des effets toxiques ont été associés à des augmentations temporaires des taux de nitrates de certaines plantes consécutives aux traitements. Il convient de respecter les recommandations relatives à l'exclusion du bétail des pâturages traités pendant une certaine période après le traitement.

Des dangers pour la vie sauvage peuvent résulter de l'usage des herbicides, mais ils peuvent également être causés par d'autres variations dans les pratiques agricoles. Les effets des herbicides sur l'habitat sont graves pour la vie sauvage et ne doivent pas être sous-estimés. Des morts se sont produites chez les abeilles à la suite de traitements de plantes mellifères à l'époque de la floraison. Il est possible que des risques analogues existent pour les insectes qui se nourrissent du nectar des fleurs. Les études relatives à d'autres insectes ont révélé des augmentations de populations plus fréquemment que des diminutions. Dans le cas de quelques espèces de pucerons, cet accroissement était associé à une réduction du nombre des prédateurs. Aucun effet n'a été observé dans les études sur les populations d'arthropodes du sol. L'accroissement d'activité des nématodes était lié aux conditions favorables pour l'invasion des plantes hôtes créées par les tératomes et autres effets dûs aux auxines. La toxicité pour les poissons et les autres organismes aquatiques s'est révelée dépendre du type de formulation utilisée pour l'épandage de la matière active. En général, la toxicité pour les poissons est basse et les applications d'herbicides à l'eau ne présentent pas de risques directs graves. Les risques d'effets secondaires doivent être évalués en liaison avec d'autres méthodes de lutte et les caractères de l'environnement des eaux à traiter. Quelques organismes aquatiques autres que les poissons peuvent être directement affectés à des doses ne dépassant pas beaucoup celles recommandées pour l'emploi des herbicides dans les eaux.

Zusammenfassung *

Toxizität und Gefährdung bei Mensch, Haus- und Laboratoriumstieren, Wild, Bienen und anderen Insekten, Bodentieren und Fischen durch MCPA, MCPB, 2,4-D, 2,4-DB, 2,4,5-T, Mecoprop und 2,3,6-TBA

* Übersetzt von F. Bär.

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werden diskutiert. Beim Menschen wurde nur ein verbürgtes Beispiel eines Todesfalles infolge Vergiftung in der westeuropäischen und nordamerikanischen Literatur berichtet. Verbürgte Fälle von sublethalen Effekten sind auch sehr selten. Jedoch werden von Zeit zu Zeit Klagen über vorübergehendes Schwindelgefühl, Übelkeit und andere Symptome von Arbeitern vorgebracht, die in der Anwendung auf dem Felde tätig sind, besonders unter Bedingungen übermässiger Spray-Inhalation. Die Möglichkeit für den Menschen, toxische Dosen dieser Verbindungen in Lebensmitteln, Milch oder Wasser zu erreichen, scheint sehr gering zu sein.

Toxizitätsteste bei einer Reihe von Laboratoriums- und Haustieren mit einem weiten Bereich von Formulierungen der Verbindungen neigen dazu, grössere Differenzen innerhalb der Formulierungen als zwischen den Verbindungen zu zeigen. Von den untersuchten Haustieren scheinen Hunde empfindlicher zu sein als andere. Die akuttoxischen Dosen der Verbindungen für Vieh und Geflügel waren höher als sie die Tiere normalerweise aus behandelten Pflanzen oder aus Wasser nach Anwendung in der Landwirtschaft aufnehmen können. Die Gefährdung des Viehbestandes durch konzentriertes Material wird hervorgehoben, aber die agressive und widerwärtige Natur der unverdünnten Chemikalien würde wahrscheinlich jedes normale Tier von der Aufnahme einer toxischen Dosis abhalten. Die Gefahr der chronischen Toxizität erwies sich auch als sehr gering und eine kontinuierliche hohe Dosierung eher über Monate als über Wochen ist erforderlich, um schwere Vergiftungssymptome zu erzeugen. Bei vielen Tieren wird ein hoher Anteil der aktiven chemischen Stoffe im Harn 24 - 48 Stunden nach der Aufnahme entleert. Sekundäre Gefährdung bei Tieren als Ergebnis von Veränderungen der chemischen Bestandteile von Nahrungspflanzen kann vorkommen; zum Beispiel war die Toxizität begleitet von einem vorübergehenden Anstieg des Nitratgehaltes gewisser Pflanzen nach dem Besprühen. Laufende Empfehlungen für die Fernhaltung des Viehbestandes von der behandelten Weide für einige Zeit nach der Behandlung sollten befolgt werden.

Eine Gefährdung für Wild besteht durch die Anwendung von Herbiziden; sie ist jedoch ebenso verbur[illegible]n mit anderen Änderungen in der Landwirtschaftspraxis. Die Effe[illegible]e der Herbizide auf dem Standort beim Wild sind ernst und sollten nicht unterschätzt werden. Tod von Bienen erfolgte nach dem Besprühen von Nektar-erzeugenden Pflanzen in der Blüte. Es ist möglich, dass eine ähnliche Gefährdung für Nektar-fressende Insekten besteht. Beim Studium anderer Insekten wurde ein Populationsanstieg häufiger als eine -abnahme festgestellt. Bei Blattläusen war dies verbunden mit der Herabsetzung der Anzahl der Raubinsekten. Keine Effekte wurden festgestellt in Untersuchungen von Populationen von Bodenarthropoden. Eine gesteigerte Aktivität durch Nematoden war verbunden mit günstigen Bedingungen für den Befall der Wirtspflanze, erzeugt durch Auxin-induzierte Teratome und

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andere Effekte. Die Toxizität für Fische und andere Wasserorganismen zeigte eine Abhängigkeit von der Formulierung der angewandten Verbindung. Im allgemeinen ist die Toxizität für Fische gering und die Herbizidanwendung zum Wasser stellt keine schwere direkte Gefährdung dar. Die Gefahren sekundärer Effekte sind in Beziehung zu setzen zu anderen Kontrollmethoden und zu den Umwelteigenschaften der zu behandelnden Wasserfläche. Ausser den Fischen können gewisse Wasserorganismen direkt beeinflusst werden bei Dosisbeträgen, die nicht sehr weit über den zur Herbizidanwendung im Wasser empfohlenen liegen.

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Toxicity and hazards to man, domestic animals, and wildlife from some commonly used auxin herbicides

By
J. M. Way *

Contents

I. Introduction 37
II. Toxicity and hazards to man 38
III. Toxicity and hazards to domestic and laboratory animals 41
a) Acute and chronic toxicity 41
b) Indirect toxicity 46
IV. Bees and other insects 48
V. Soil animals 49
VI. Fish and aquatic organisms 50
VII. Wildlife in general 53
Summary 54
Résumé 55
Zusammenfassung 56
References 58

I. Introduction

This review has been compiled from searches in the literature for references to the toxicity and other hazards that may arise from the use of auxin herbicides, and specifically of formulations of MCPA (4-chloro-2-methylphenoxyacetic acid), S.S. 2,4-D (2,4-dichlorophenoxyacetic acid) and of their butyric acid analogues MCPB and 2,4-DB, of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), mecoprop [± α-(4-chloro-2-methylphenoxy) propionic acid], and 2,3,6-TBA (2,3,6-trichlorobenzoic acid).

Assuming 70 percent of the eight million acres of cereals grown in the United Kingdom to be treated annually with herbicides (Wood-

* Monks Wood Experimental Station (The Nature Conservancy), Abbots Ripton, Huntingdon, England.

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FORD 1964), it is apparent that the tonnage (circa 2,500 tons) of the compounds mainly used for this purpose is similar to the total tonnage of insecticides and fungicides used annually on all crops in the U.K. (circa 2,660 tons) (STRICKLAND 1966). In 1962, DADD (1962), from a survey of eastern England, found that over 90 percent of cereal fields treated with herbicides were treated with auxin compounds based on chlorinated phenoxy acid derivatives, notably salts of MCPA and to a lesser extent of mecoprop. Although the use of straight formulations of MCPA has been gradually declining (WOODFORD 1964) in favour of mixtures of compounds, many of these mixtures still include MCPA or other phenoxy compounds and increasingly include 2,3,6-TBA. In addition to its agricultural use on arable and pasture crops, 2,4-D together with 2,4,5-T, is used, or is likely to be used on an increasing scale, for scrub control and control of unwanted broad-leaved tree species in conifer plantations. 2,4-D is also used to a limited extent for control of submerged aquatic weeds. On a world-wide basis 2,4-D is probably used to a greater extent than any other herbicide and this is reflected in the greater number of references to this compound. The extensive use of a single biologically active family of compounds clearly presents a considerable potential hazard in respect to their direct and indirect toxicities to animals including man. It is a measure of the comparative safety of the auxin herbicides that, in spite of their increasing use throughout the U.K. since the mid-1940's, they continue to be applied much in the same routine way as are inorganic fertilizers, and with as little regard for any possible toxic hazard.

During the many years of their use throughout the world there have been notably few authenticated incidents of poisoning of domestic animals or wildlife resulting from the proper application of these compounds. The fact that fully authenticated incidents of poisoning have not been reported cannot be taken as an absolute guarantee of safety, particularly with regard to indirect or chronic toxicity, because of the frequent difficulties of attributing the underlying cause of an illness or of death, especially in wild animals. Nevertheless all the direct and circumstantial evidence to hand at the present time seems to indicate that incidents of poisoning have been extremely few and generally under exceptional circumstances.

II. Toxicity and hazards to man

The principal routes of toxicity to man are either orally or by inhalation; there appears to be little hazard of transport through the skin although individual allergies can develop leading to dermatitis (VALLET 1965). Eyes may be directly but are usually only temporarily affected. Hazards to man may occur from the concentrated chemical before dilution, from inhalation of spray or dust during application, or from ingestion of the chemicals in food or in water. Because the great-

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est hazards are from the concentrated chemical and because man is handling the chemicals in this form at all stages from manufacture to dilution, it follows that he is at greater potential risk than any other organism. However, there are very few reports in the literature of tests or incidents of poisoning of man by these compounds; the majority of these reports refer to accidental poisoning of children. As a result it is now generally accepted that auxin-type herbicides do not present a direct toxicity hazard to man (BARNES 1955) when correctly handled or used for weed control.

KRAUS in 1945 (in KEPHART 1945) reported that he had taken 0.5 g. of 2,4-D *per diem* for 21 days with no demonstrable ill-effects. A clinical study was made in Denmark by NIELSEN *et al.* (1965) on a 23-year old man who had committed suicide by apparently drinking 125 ml. of 50 percent w/v 2,4-D dimethylamine salt. The total weight of 2,4-D in his body was calculated as being not less than six g. (the equivalent of 80 mg./kg.), about 10 percent of the total weight of active material ingested. The principal damage appeared to be to nerve tissues and the central nervous system.

VALLET (1965) gives acutely toxic oral doses of 2,4-D to man of 50 to 500 mg./kg. body weight, of 2,4,5-T of 500 to 1,000 mg./kg., and of MCPA of 50 mg./kg. No indication is given of the source of these figures which are probably extrapolated from data on laboratory animals. FREIREICH *et al.* (1966) have shown that a number of drugs, on a mg./kg. basis, are more toxic in man than in the mouse, when used as a laboratory test animal, by a factor of 10 to 15. There are clearly dangers therefore in assuming that a toxic dose to man is the same as the toxic dose to a laboratory animal multiplied by some factor based on the mean differences in weight. In fact these latter authors show for a number of anticancer agents that a very much more accurate assessment of the ratio of animal to human toxicity can be obtained on a mg./m.^{2} of body surface area, than on a mg./kg. basis. For this reason VALLET's (1965) figures may be suspect, particularly in respect of the range of 50 to 500 mg./kg. for 2,4-D, although the lower figure would be of the correct order of magnitude when compared to the residue of 80 mg./kg. found by NIELSEN (1965) in his suicide case. Nevertheless, this figure represented the amount actually found and it is likely that a greater proportion of the total 2,4-D ingested was necessary to produce death. In this context it is noteworthy that the acute oral LD_{50}'s to rats of the most common organochlorine insecticides range between 10 and 135 mg./kg. (MARTIN 1963).

EDWARDS and RIPPER (1953) have discussed the hazard to operators from inhalation of sprays or aerosols during application of herbicides with particular reference to methods of protection. MONARCA and DI VITO (1961) have described a clinical study of an acute case of accidental poisoning of a man in Italy. In this instance a farmer became ill after applying a 40 percent aqueous solution of 2,4-D by handpump

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against the wind. He was admitted to hospital, suffered a relapse after 18 days, and recovered sufficiently to be discharged after 40 days. Initial symptoms of muscular weakness, vomiting, perspiring freely, and oliguria were noted in the field whilst a diagnosis of bradycardia, respiratory difficulties, and urinary abnormalities was made after admission to hospital. However the authors report that the case was exceptional. FETISOV (1966) has reported similar field symptoms in Russian workers engaged in field applications of 2,4-D. This author concluded that a range of formulations of 2,4-D was "highly toxic to animals in different ways of introduction." Whilst reports of minor discomfort following exposure to auxin sprays during field application are rarely reported in scientific literature, there is no doubt that a proportion of workers so exposed do suffer a degree of transitory discomfort. Whether this is of any significance as a long-term toxic hazard has not been determined for man.

From the very nature of their use it is unlikely that auxin herbicides will appear as significant residues in food crops. WILLIAMS (1964) was unable to detect any residues of auxin herbicides in a number of total diet samples down to the limit of sensitivity (0.01 p.p.m.) of his analytical techniques. DUGGAN and WEATHERWAX (1967) calculated pesticide chemical residues in 'total diet' samples collected on 46 days in 25 American cities during a 699 day period from June 1964 to April 1966. Each sample represented the total amount of food and drink consumed by one person over a two-week period. The total samples represented in all a food and drink supply sufficient for 644 days. Herbicide chemicals were found infrequently and averaged about 0.01 mg./day of which one third was 2,4-D, and half was MCPA and pentachlorophenol (PCP) combined. 2,4-D was found in oils and fats (0.001 mg. in 1964/65) and sugars and sugar products (0.004 mg. in 1964/65, 0.002 mg. in 1965/66), whilst MCPA was found in grain and cereals (0.002 mg. in 1964/65), in dairy products (0.003 mg. in 1965/66), and in leafy vegetables (0.001 mg. in 1965/66). These amounts are substantially below the limits set for acceptable daily intake by the World Health Organization and United Nations committees. It seems probable, therefore, that toxic hazards from auxin herbicide residues in food are very small. FAUST (1964), in a survey of water pollution hazards to man from organic pesticides, came to the conclusion that there did not appear to be danger to health at the present time from the background concentration of pesticides believed to be in ground and surface water. However, 2,4-D could persist in lake water and bottom mud for long periods under certain environmental conditions. Work in Russia, quoted by FAUST, suggested that the threshold taste and odour concentrations auxin compounds, especially of phenolic derivatives such as 2,4-D, that would prove unacceptable to the consumer were very considerably below the threshold concentrations for toxic effects. A particular risk might be supposed to lie in contaminated milk

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drawn from cows feeding in treated pasture, but no residues of either 2,4-DB or 2,4-D were found in the milk of cows that had been fed these compounds (GUTENMANN *et al.* 1963a and 1963b). The beasts were fed 5.0 p.p.m. (based on a daily ration of 50 pounds) for a number of days; the limit of detection for 2,4-DB was 0.2 p.p.m. and for 2,4-D 0.1 p.p.m. Whereas milk might be a source of contamination these results indicate that there is no apparent hazard.

III. Toxicity and hazards to domestic and laboratory animals

a) Acute and chronic toxicity

The toxicity of agricultural chemicals to land fauna is normally quoted in terms of the dose that kills 50 percent of a population of test animals (LD_{50}). Whilst this figure gives a useful indication of the comparative toxicities of different compounds to a given test species, the figures obtained in different tests may be influenced by a number of factors. Thus the age and sex of the test animal, method of dosing, and general conditions of the test may have an important bearing on the susceptibility of the animal to the compound being studied. The formulation of the active compound has a considerable influence: for instance 2,4-D acid has an LD_{50} to rats of 375 mg./kg. but the sodium salt has an LD_{50} of 805 mg./kg., the propylene glycol butyl ether ester of 570 mg./kg., and the iso-propyl ester of 700 mg./kg. (ROWE and HYMAS 1954). It should be noted, however, that BJÖRKLUND and ERNE (1966) do not regard these differences as being appreciable. In addition, the LD_{50} for different test species may vary quite widely: for example, 2,4-D acid has an LD_{50} of 375 mg./kg. for rats, of 100 mg./kg. for dogs, 469 mg./kg. for guinea pigs, and 541 mg./kg. for chicks (ROWE and HYMAS 1954).

Bearing in mind these reservations, the acute oral LD_{50} to rats of the acid formulations of the compounds under consideration are: 2,4,5-T 300 mg./kg., 2,4-D 400 mg./kg., MCPB 680 mg./kg., MCPA 700 mg./kg., 2,4-DB 700 mg./kg., 2,3,6-TBA 705 mg./kg., and mecoprop 930 mg./kg. (WOODFORD and EVANS 1965). Thus, 2,4-D and 2,4,5-T fall in the moderately toxic and the other five compounds in the slightly toxic class according to the widely accepted classification of toxicity of chemicals quoted by FRAZER (1963). In a comparison of the relative toxicities of a number of commercial formulations of 2,4-D and 2,4,5-T, ROWE and HYMAS (1954) concluded that the 'inert' constituents of the formulations did not add to the toxicity of the active compound, nor did they exact any potentiating effect. Similar conclusions were reached by DALGAARD-MIKKELSEN and POULSEN (1962) on the basis of this and other work. However, it is possible for surfactants and other additives to enhance the toxicity of active compounds. Whilst there is some evidence with insecticides that mixing

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of compounds leads to synergistic effects on the acute toxicity of the constituent compounds (KEPLINGER and DEICHMANN 1967), effects of this sort have not been reported for herbicide mixtures in common use. However, the possibility of their occurring should not be overlooked, nor, also, the possibility of antagonism where the effect of one compound upon another in a mixture is to depress its biological activity.

Descriptions of the clinical symptoms of poisoning by MCPA, 2,4-D, and 2,4,5-T of a range of experimental animals have been summarized by ROWE and HYMAS (1954) and more recently reviewed with comments by DALGAARD-MIKKELSEN and POULSEN (1962). BJORKLUND and ERNE (1966) have subsequently further reviewed and reported on their own results on the effects of feeding 2,4-D to calves, pigs, rats, and chickens. A range of symptoms was produced that was generally similar to those described by the previous authors. ERNE (1966a and b) studied the distribution and elimination of 2,4-D and 2,4,5-T in these animals. Amine and alkali salts of both compounds were readily absorbed and completely distributed in the body, but 2,4-D ester was incompletely absorbed and reached only a low level in the plasma and tissues. The highest tissue levels of the two compounds were found in liver, kidney, spleen, and lungs and the levels found in these organs sometimes exceeded the level found in the plasma. In blood cells some 10 to 20 percent of the plasma level was found. Penetration of 2,4-D into placental tissue of pigs was recorded but there was little or no evidence of penetration into adipose tissue or the central nervous system. Elimination of the compounds was rapid, the plasma half-life being about three hours in rats, eight in calves and chickens, and 12 in pigs. The tissue half-life values ranged between five and 30 hours. No retention of the compounds was noted in the tissues. There was no accumulation after repeated dosing and in pigs there was an increase in the rate of elimination after repeated administration. In all species the main excretory route was *via* the kidneys. KHANNA and FANG (1966) traced the metabolism of C^{14}-labelled 2,4-D in rats dosed at rates from one to 100 mg./animal. Radioactivity was found in all the organs studied together with some accumulation as early as one hour after dosing. At the one mg. dose rate a concentration peak of radioactivity was demonstrated after six to eight hours but decreased thereafter and was non-detectable by 24 hours. At the 80 mg. dose the peak occurred at eight hours and persisted for 17 hours. Extracts of the tissues were shown to contain mainly unchanged 2,4-D residues. No radioactivity was found in the expired carbon dioxide, but elimination in urine and faeces was dose dependent. At the one to 10 mg. doses 93 to 96 percent of the ingested 2,4-D was excreted unchanged in the urine in the first 24 hours. At the 20 to 100 mg. doses greater amounts of 2,4-D were found in the second 24 hour period after dosing, with a linear decrease in percentage recovery with increase in dose. In experiments with cattle GUTENMANN *et al.* (1963a and b) were un-

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able to detect any residues of 2,4-DB or 2,4-D in milk or faeces of cows fed five p.p.m. of either compound in a 50 pound daily ration. In these experiments there was no evidence of beta-oxidation of 2,4-DB to 2,4-D. Disappearance of 2,4-D was thought to occur as a result of dilution in the rumen, some absorption on the gut wall, and by decomposition. In subsequent experiments BACHE *et al.* (1964) and ST JOHN *et al.* (1961) studied the fate of MCPA, MCPB, 2,4,5-T, and a number of other herbicides in cattle. All the MCPA fed to a single steer (113.5 mg. single dose based on five p.p.m. of a 50 pound daily ration) was accounted for in its urine over the four days after administration. No MCPB was found in the urine of MCPB-dosed cows (total dose of 56.75 mg. and 113.5 mg. based on 2.5 and 5.0 p.p.m. of a 50 pound daily ration) on the first day after administration, but subsequent recoveries in their urine showed that 7.2 to 9.2 percent of the MCPB was converted to MCPA. Analysis of MCPB itself, however, was said to be unsatisfactory. It is of interest that a similar conversion of 2.4-DB to 2,4-D can be performed by bluegill sunfish (*Lepomis machrochirus*) (GUTENMANN and LISK 1965). Urine and milk samples were also analysed from cows fed a total of 454 mg. of 2,4,5-T over a period of four days. It was shown that the compound was excreted exclusively in the urine over a period of six days, a total of 430.7 mg. being recovered. CLARKE *et al.* (1964) investigated the fate of C^{14}-labelled 2,4-D in sheep fed a dose of four mg. of 2,4-D/kg. calculated as the minimum daily dose likely to be ingested from grazing treated pasture. The level of radioactivity in the blood rose to a peak 1.5 hours after dosing but fell to the normal background level in 24 hours. All the material was excreted within 72 hours, with over 98 percent in the urine and only 1.5 percent in the faeces. BALAYANNIS *et al.* (1965) dosed a rabbit and six mice with 2,3,6-TBA and showed that a proportion of the compound was recovered after passing through the animals. These authors discussed the significance of biologically active residues of auxin compounds in animal excreta that might become incorporated in manure or straw. It was shown that 2,3,6-TBA residues in particular could remain active for a period of months and affect susceptible crops to which the contaminated manure was applied. The same hazard does not normally exist with the phenoxyacetic acid derivatives, where the compounds are broken down and become biologically inactive in a relatively short period of time. However, LISK (1966) has pointed out that the excretion of 2,4-D in the urine of cows does present the admittedly remote possibility of active 2,4-D being transferred from a treated pasture to a susceptible crop.

MITCHELL (in KEPHART 1945), DALGAARD-MIKKELSEN *et al.* (1959), and GOLDSTEIN and LONG (1960) all reported that there were no apparent ill-effects in cattle, sheep, or horses from grazing pasture sprayed at herbicidal or two times herbicidal rates of 2,4-D or MCPA. GRIGSBY and FARWELL (1950) (in SPRINGER 1957) reported that there

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was no significant difference in the amount of feeding of horses, cows, sheep, and pigs in untreated plots or plots sprayed with the sodium salt or the isopropyl ester of 2,4-D, or the isopropyl ester of 2,4,5-T. However, there did appear to be less feeding in plots sprayed with the alkanolamine salt of 2,4-D. There was no effect on milk production of cows feeding on sprayed vegetation. GOLDSTEIN and LONG (1960) found no ill-effects on two cattle from adding 0.25 pints of a 1.5 percent w/v 2,4-D/2,4,5-T mixture to every five gallons of their drinking water for 41 days. These authors also reported spraying the skins of a calf, of a cow, of sheep, and of pigs with doses ranging from 0.002 to 0.008 pounds of 2,4-D or 2,4-D/2,4,5-T mixture, with no ill-effects. These dose rates would be of the order of those that might occur in an instance of spray drift.

DOBSON (1954) sprayed MCPA, 2,4-D, or 2,4,5-T on grassed chicken runs daily for 14 days at normal and ten times normal dose rates. 2,4,5-T significantly reduced egg production and the weight of the birds; MCPA and 2,4-D also affected egg production, mainly in the second week of spraying or during the week after spraying had stopped. In all instances there was no effect on the fertility of the eggs and all the progeny reared well, although the dose rates and frequency of application in this trial were much more severe than are likely to be found in practice. ERNE (1960) showed that some of the 2,4-D fed to hens could be excreted in their eggs. DUNACHIE and FLETCHER (1967) injected hen's eggs with MCPA, 2,4-D, MCPB, 2,4-DB, mecoprop, and 2,3,6-TBA amongst a range of other herbicides. Dose rates were 10, 100, and 200 p.p.m. equivalent to 0.5, 5, and 10 mg./egg. The percentage hatch was recorded. At the lowest dose there was 100 percent hatch from the MCPA- and MCPB-treated eggs; 90 percent from the mecoprop- and TBA-treated eggs, and 80 percent from the 2,4-D-treated eggs. At the highest dose there was 80 percent hatch from mecoprop, 50 percent from 2,4-D and TBA, and 20 percent from MCPA and MCPB. None of the chicks that hatched was deformed although some feather blanching was noted from the MCPA, MCPB, 2,4-DB, and mecoprop treatments. ROBERTS and ROGERS (1957) reported on various feeding experiments on turkeys with alfalfa sprayed with a low volatile ester of 2,4,5-T at herbicidal rates. No deleterious effects were noted. Calculations were quoted to show that for a one kg. chicken to acquire a lethal dose of 2,4-D from an application rate of one pound/acre, the bird would have to consume in two days all the 2,4-D applied to the vegetation over an area of 72 square feet. Similar calculations by ROWE and HYMAS (1954) for cattle suggested that for a 770 pound animal to acquire an acutely toxic dose of 2,4-D of approximately 1,000 mg./kg., it would have to graze completely and ingest all the 2,4-D from an area of 0.17 acre (one pound/acre ≡ 10.41 mg./ft.^{2} ≡ 93.7 mg./yard^2 ≡ 112.1 mg./m.^{2}).

Accounts are given of direct oral dosing or dermal applications of

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auxin herbicides to a variety of domestic animals by KEPHART (1945) (cow), ROWE and HYMAS (1954) (laboratory animals and cattle), DALGAARD-MIKKELSEN *et al.* (1959) (heifers), PALMER (1963) (cattle), PALMER and RADELEFF (1964) (sheep and cattle), CLARKE *et al.* (1964) (sheep), and STRACH and BOHOSIEWICZ (1964) (pigs). PALMER (1963) gave daily oral doses of 2,4-D alkanolamine salt to steers for five days in every seven. He recorded signs of poisoning in animals dosed at 250 mg./kg. after 15 administrations as opposed to 86 administrations of 100 mg./kg.; at 50 mg./kg. no ill-effects were recorded over a period of 112 administrations. From these results he concluded that although animals could probably ingest enough 2,4-D from concentrated solutions at any one time to produce illness or death, the chronic toxicity of the compound was sufficiently low to make it unlikely that an animal would pick up enough of it over a period of time to cause any serious ill-effects. Further work by PALMER and RADELEFF (1964) using single animals gave the following results:

1. Sheep tolerated 481 daily doses of 100 mg./kg. of the alkanolamine salt or propylene glycol butyl ether ester of 2,4-D.
2. Cattle suffered from chronic tympanites after 88 daily doses of 100 mg./kg. of the alkanolamine salt of 2,4-D. One animal died after 34 daily doses of 200 mg./kg.
3. Sheep tolerated 481 daily doses of 100 mg./kg. of the triethylamine salt of 2,4,5-T but succumbed to 369 doses of 100 mg./kg. of the propylene glycol butyl ether ester.
4. Sheep were killed by 383 daily doses of 100 mg./kg. of MCPA amine.

The toxicity of 2,3,6-TBA in these trials was said to be slightly greater than that of 2,4-D or 2,4,5-T. STRACH and BOHOSIEWICZ (1964) reported that no abnormal behaviour in pigs had been noted following 40 daily doses of 15 to 100 mg./kg. of 2,4-D, nor from single doses of 2,4-D of 200 to 800 mg./kg.

In short term trials by BJÖRKLUND and ERNE (1966), calves and pigs showed definite though reversible symptoms of poisoning after single doses of 2,4-D of 200 and 100 mg./kg., respectively. Rats and fowls did not show any sign of distress after single doses of 100 and 300 mg./kg., respectively, and fowls tolerated daily doses of 300 mg./kg. daily in their feed for several weeks without visible effects. Repeated daily doses of 50 mg./kg., however, led to toxic symptoms in some pigs. In longer term studies (ERNE 1966a), five young pigs were fed 500 p.p.m. of 2,4-D for up to 12 months but, although various toxic effects were noted and their growth rate was affected, none of the animals died. When 2,4-D was fed to a sow throughout gestation and for a further six weeks, 10 of the 15 underdeveloped and apathetic piglets she produced died within 24 hours and the mother subsequently had to be slaughtered because of abnormalities that developed in her spine. Heavy dosing of pregnant rats, however, with 1000 p.p.m. of

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2,4-D in their drinking water over 10 months and of their off-spring for up to two years, whilst leading to retarded growth and increased mortality, did not produce unequivocal signs of toxicity. Continued administration of 500 p.p.m. of 2,4-D in feed or 1,000 p.p.m. in the drinking water of fowls led to reduced egg production and kidney abnormalities. These results led the authors to conclude that the chronic toxicity of 2,4-D to the species studied was moderate. They were, however, concerned about the mortality of new-born piglets, with evidence of movement of 2,4-D through the placental tissues, and the reduced egg production in fowls which they thought might indicate a possible interference with reproductive processes.

In general the findings of other workers support these conclusions on acute and chronic toxicity. In all the work quoted the amounts administered to the test animals for effect, have been well in excess of the amounts they might be expected to pick up from a treated pasture, or in feed derived from crops that had at some time been treated with auxin herbicides at normal dose rates.

b) Indirect toxicity

Indirect effects of herbicides on grazing animals have been associated with increased toxicity of toxic plants, increased palatability of normally non-palatable toxic plants (e.g., ragwort, *Senecio jacobaea*), and induced toxicity in normally nontoxic plants (e.g., temporary increases in nitrate content) (WILLARD 1950). However, FERTIG (1953) claimed that, up to 1953 in America, in all cases where poisoning of livestock from herbicides had been reported, the effects noted could be attributed to some other cause.

Examples have been given by WILLARD (1950) of cattle eating wild cherry (*Prunus serotina*), of pigs eating Cocklebur (*Xanthium* sp.), and of lambs eating thistles after herbicidal treatment with auxins. Instances have been reported of ragwort becoming 'sweeter' from two or three days after application and being preferentially grazed by cattle for a short period. GRIGSBY and BALL (1952) and LYNN and BARRONS (1952) investigated the hydrocyanic acid (HCN) content of the leaves of wild cherry from untreated trees and trees treated with 2,4-D and 2,4,5-T. Their conclusions were that the foliage was no more toxic to cattle after treatment and that there might even be less HCN in the leaves of the treated trees than in those of the untreated ones. BUCK *et al.* (1961) fed the alkaloid-containing plants *Delphinium barbeyi* (tall larkspur) and *Helenium hoopscii* (sneeze weed), after treatment with 2,4-D ester or 2,4,5-T ester, to calves and ewes. No increased toxicity of the plants attributable to application of the herbicides was noted. WILLIAMS and CRONIN (1963) analysed *D. barbeyi*, treated with 2,4,5-T amine at various growth stages, and showed that the alkaloid content of the plants was increased for several

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weeks after treatment at the vegetative and early bud stages. It was noted, however, that the bitter taste of the alkaloids might make the treated plants even less palatable to animals than untreated ones.

SWANSON and SHAW (1954) showed that 2,4-D affected the HCN content of Sudan grass (*Sorghum vulgare* ssp. *sudanense*). Initially there was a decrease in the content of HCN but four days after treatment there was an increase over the controls which was maintained for a further 12 days. Similar effects were shown to occur with the nitrate content of leaves. BUCK *et al.* thought that there might be a relationship between HCN and nitrate metabolism in Sudan grass, an increase in one leading to a decrease in the other.

The clinical aspects of nitrate poisoning in stock, conditions under which nitrates are likely to accumulate in the leaves of certain plants, and lists of these plants have been reported by BRADLEY *et al.* (1940), DAVIDSON *et al.* (1941), GILBERT *et al.* (1946), CASE (1957), and SUND *et al.* (1960). The toxic effects of nitrate are caused by a reduction of nitrate to nitrite and the conversion by nitrite of haemoglobin in the blood to methoglobin: the animal dies from asphyxia. Intravenous injection of methylene blue in doses of two g./500 pounds of body weight gives immediate relief. Nitrate in plants is generally present in the form of potassium nitrate and increases in nitrate content have been associated with drought conditions and high soil nitrogen (GILBERT *et al.* 1946, CASE 1957). SUND *et al.* (1960) noted a high nitrate content in *Urtica* spp. and *Rubus* spp. after heavy rains, followed by preferential grazing of these and other weed species by cattle. A number of abortions in these cattle was correlated with occurrence of high nitrate rather than grazing of the weed species *per se*. Recent increases in vitamin A deficiency in North American ruminants has been associated with ingestion of nitrates occurring in herbicide-treated plants by PHILLIPS (1964).

The accumulation of nitrates in the leaves of sugar beet is well known (*e.g.*, SAVAGE 1949). Increased levels of nitrate in the leaves of this crop as a result of herbicide application have been reported by WILLARD (1950), STAHLER and WHITEHEAD (1950), and WHITEHEAD *et al.* (1956). Isolated incidents have been reported of nitrate poisoning of cattle in America as a result of feeding on sugar beet that had previously been sprayed. In one incident in N. Dakota, the nitrate content of sugar beet leaves after spraying was found to vary from 1.81 to 8.77 percent of the dry weight, as against 0.22 percent for untreated plants and a toxic level of 1.5 percent (STAHLER and WHITEHEAD 1950). Studies on forage crops (BING and MCELROY 1953) and on a range of weed species (FRANK and GRIGSBY 1957) have shown which of these may contain high levels of nitrates after auxin application. Cell-free extracts of maize and cucumber from plants that had been previously sprayed with 10 and 100 p.p.m. of 2.4-D were investigated by BEEVERS and HAGEMAN (1962). The level of nitrate reductase was

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increased in maize but reduced in cucumber. Studies on the formation and breakdown of nitrates in plants (FERTIG 1952, FREIBERG and CLARK 1952, WHITEHEAD *et al.* 1956) have shown that 2,4-D causes more rapid increases in nitrate content than MCPA, that levels rise to a peak soon after spraying and subsequently decrease with time, and that increases in light intensity hasten decreases in nitrate content.

It is clear from these reports that nitrate poisoning in stock does occur from time to time and that it is possible for the hazard to be increased by application of auxin herbicides to nitrate-accumulating plants.

IV. Bees and other insects

Herbicides affect bees (*Apis mellifera*) and other insects if they kill the plants on which the insects feed. In addition, WAHLIN (1950) has reported that 2,4-D and MCPA were toxic to bees, not only from visiting the flowers but also as a result of drinking contaminated water trapped on treated plants. ANTOINE (1966) reported that MCPA paralysed and killed bees which ingested doses that corresponded to those recommended for weed control. Other workers have reported effects on bees after application of auxin herbicides to plants in flower but not at other times (HARAGSIMOVA 1962, PALMER-JONES 1964). PALMER-JONES (1964) and ANTOINE (1966) have suggested that 2,4-D might have some effect on nectar which made it toxic to bees. KING (1960a) has shown that radioactive 2,4-D can be translocated to the nectar of *Poinsettia* and red clover plants and may be detectable there for two to three days after treatment. Feeding trials of auxin herbicides to bees have been reported by GLYNNE JONES and CONNELL (1954), PALMER-JONES (1960), KING (1960b), and BYRDY (1962). PALMER-JONES (1964) found no effect on bees that had been directly dusted with 2,4-D or when they were made to crawl through 2,4-D dust in order to enter the hive. GLYNNE JONES and CONNELL (1954) classed 2,4-D and MCPA as stomach/contact poisons of low toxicity to bees, with LD_{50} values of 0.105 mg. compared to insecticides in the range 0.00004 to 0.002 mg. BYRDY (1962), on the other hand, reported total mortality of bees within four days of feeding 30 μg. of 2,4-D and 10 percent mortality within three days rising to 20 percent in five days of feeding 20 μg. JOHANSEN (1959) reported that 2,4-D and related compounds were not toxic to bees, except when formulated as the alkanolamine salt or the isopropyl ester. HARAGSIMOVA (1962) found that a 30 percent calcium/potassium salt formulation of MCPA was toxic to bees and caused mortality up to 13 days after ingestion. However, part at least of the loss was attributed to cresol impurities, which led to loss of smell amongst the bees with consequent loss of sense of direction.

Occasional observations on other insects have been reported.

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MELLANBY *et al.* (1959) observed that a spray of mecoprop on oats at the time of a heavy frit fly attack had no effect on the fly (*Oscinella frit*). MAXWELL and HARWOOD (1960) treated broad bean (*Vicia faba*) plants with sublethal doses of 2,4-D and recorded a marked increase in the reproduction of the pea aphid (*Macrosiphum pisi*) feeding on them. The longevity of adult aphids was unaffected. ROBINSON (1959) also recorded increased fecundity in another pea aphid (*Acyrthosiphon pisum*) after caging on broad bean plants treated with 2,4-D. ADAMS (1960) and ADAMS and DREW (1965) showed that the application of 2,4-D amine could enhance aphid infestation in New Brunswick grain fields, probably as a result of depressing the activities of coccinellid beetles predating on the aphids. In laboratory experiments with coccinellid larvae treated with 2,4-D amine, there was a fourfold increase in mortality and an increase in time to pupation. There was little mortality amongst the adult beetles, which usually recovered after a few hours inactivity. ISHII and HIRANO (1963) concluded that increases in the growth rate of the larvae of the rice stem borer (*Chilo suppressalis*) feeding on 2,4-D treated rice plants, was a consequence of increased nitrogen content of the plants rather than a direct effect of the chemical itself.

In general, it appears that there is a real hazard to bees—and possibly other nectar feeding insects—from applications of auxin herbicides to plants in flower. Otherwise there would seem to be little hazard to insects from direct toxicity of the compounds at normal herbicidal rates of application. However, the work of ADAMS and DREW (1965) does suggest that some insects may be more susceptible at particular stages of their life cycle than at others.

V. Soil animals

Amongst soil arthropods, DAVIS (1965) was unable to show any differences in populations of *Acari or Collembola* species between untreated plots and plots that had been sprayed with MCPA in 10 out of 13 years. DAVIS (1965) was unable to find any reports of significant effects on soil animals in other experiments of a similar nature. BOLLEN (1961) concluded that auxin herbicides, based on phenoxyacetic and propionic acid, were the most susceptible to breakdown by microorganisms of the many pesticides applied to the soil. The importance of soil microorganisms in the breakdown of these herbicides is well known from the work of AUDUS (1964) and others. WEBSTER (1967) has briefly reviewed the literature on the influence of plant growth-regulator-auxin herbicides on the host/parasite relationships of nematodes, in which 2,4-D has been shown to increase nematode reproduction in plant callus cultures. In addition, plant cell hypertrophy and proliferation, which is a common effect of 2,4-D in many plants, provides highly suitable conditions for development of nematodes. In

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this way susceptibility of a normally nematode-resistant variety of oats could be induced, although there did not appear to be any greater susceptibility of a non-resistant variety.

VI. Fish and aquatic organisms

Under field conditions the toxicity of a pesticide in water is affected by a number of factors in addition to those that affect its performance on land. Thus acidity, hardness of the water, and the sorbent qualities of suspended organic matter in the water may directly effect the toxicity. The trophic nature of the ecosystem, the oxygen status of the water in respect of both producers and demand, and the amount of movement of water both within the system and in terms of flow will affect the concentration of the chemical, its persistence, and its possible toxic side effects. Because of these, and many other interacting factors, the toxicity of a given formulation of a given chemical compound to an individual species will vary under field conditions depending upon the nature of the water body and the immediate environment. For this reason toxicities to fish and aquatic organisms are usually estimated in terms of median tolerance limit for exposure to a given concentration of the pesticide, for a given length of time (TLmx).

In addition to direct or indirect toxicity, the effects on aquatic organisms of the removal of the substrate that gives them food and shelter must also be considered. For instance, in one of the Tennessee Valley Authority's reservoirs two applications of 2,4-D controlled considerable acreages of Eurasian water milfoil (*Myriophyllum spicatum*). The eradication of the plant eliminated the substrate that might have been colonized by large populations of epiphytic insects such as the larvae of midges, mayflies, and dragonflies (Smith and Isom 1967). Although under some conditions this might have a very serious effect, it has been shown by Way *et al.* (1968) in an ecological investigation of the use of paraquat in lakes, that although individuals and populations of animals may be severely affected in the weeks after application of a herbicide, the species affected may be present again in the season after treatment. It has also to be recognized that very heavy infestations of submerged or floating aquatic plants may interfere with the passage of nutrients and considerably reduce the temperature and dissolved oxygen values of the water (Fish 1966). Thus, any possible hazards from the use of a herbicide may be outweighed by the advantages gained from the removal of the vegetation.

The danger of significant amounts of 2,4-D appearing in ground water or streams as a result of local applications seems to be slight. Aldhous (1967) measured residues of 2,4-D in drainage channel water after aerial spraying of a Scottish forest at four pounds/acre active ingredient in 12 gallons of water. Up to two p.p.m. of 2,4-D was measured in the seven days after application but none was detectable

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by 28 days. BROWN and NISHIOKA (1967) analysed samples of a water-suspended sediment mixture from 11 streams in the western United States at monthly intervals. Amongst the analyses made were gas chromatograph determinations for 2,4-D and 2,4,5-T. Neither compound was found in any of the samples. The absence of the compounds was attributed to some extent to their susceptibility to degradation.

Reviews of toxicity hazards to fish of a range of pesticides, including auxin herbicides, have been made by BAUER (1961), BANDT *et al.* (1962), and COPE (1965 and 1966). COPE (1966) noted that variations in formulation gave rise to greater differences in toxicity than the differences in toxicity between the basic compounds. Ester formulations were often more toxic than amine or metallic salt formulations. Similar observations were made by LHOSTE (1959) who reviewed effects on a number of crustaceans, aquatic insects, and molluscs.

Trout (*Salmo trutta*) are normally regarded as being amongst the most sensitive fish to water pollution. ALABASTER (1958) has given median tolerance limits for 24 and 48 hour (TLm_{24} and TLm_{48}) exposures of trout to 2,4-D or 2,4,5-T, or to mixtures of these two compounds, of 9.5 to 250 p.p.m., depending on formulation, compared to 1,150 to 2,000 p.p.m. for sodium chlorate or 0.005 p.p.m. for phenyl mercuric acetate. HOLDEN (1964) devised a formula for comparing the likely toxic hazards to trout from a number of pesticides [1] applied at agricultural rates. The following comparative estimates of hazard were given: aldrin = 70, PCP = 7, MCPA = 1.5, 2,4-D = 1, 2,4,5-T = 0.5, paraquat = 1/12, simazine = 1/27, diquat = 1/40, dalapon = 1/46, TCA = 1/120, and aminotriazole = 1/150. It should be emphasized that these figures refer to agricultural rates of application: hazards from the concentrates might be different.

Perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) are unlikely to be affected by MCPA, 2,4-D, or 2,4,5-T (BANDT 1957) at rates of application used for aquatic weed control, although a commercial mixture of 2,4-D and 2,4,5-T was more hazardous. In later trials BANDT *et al.* (1962) found threshold values for toxicity to perch and roach of MCPA of 200 to 215 mg./litre, of 2,4-D of 75 mg./litre, of 2,4,5-T of 55 to 60 mg./litre, and of 2,4-D + 2,4,5-T mixtures of 5 to 12 mg./litre. These results show rather greater toxicities of 2,4-D and 2,4,5-T compared to MCPA than were shown in HOLDEN's (1964) calculations (see above). The much enhanced toxicity of the 2,4-D + 2,4,5-T mixtures may have been an effect of the particular formulations used, or

[1] Aldrin = 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*exo*-1,4-*endo*-5,8-dimethanonaphthalene; PCP = pentachlorophenol; paraquat = 1,1'-dimethyl-4,4'-bipyridylium dichloride; simazine = 2-chloro-4,6-bisethylamino-1,3,5-triazine; diquat = 9,10-dihydro-8a,10a-diazonaiaphenanthrene dibromide; dalapon = sodium 2,2-dichloropropionate; TCA = trichloroacetic acid; and aminotriazole = 3-amino-1,2,4-triazole.

DOW 436204

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could demonstrate a synergistic effect of one compound on the other. DAVIS and HARDCASTLE (1959) established median tolerance limits over a 24 hour period (TLm_{24}) for bluegill sunfish (*Lepomis macrochirus*) to a number of herbicides. Values obtained when the compounds were added to relatively pure water were 2,4-D = 39 p.p.m., MCPA = 20 p.p.m., 2,4-DB = 20 p.p.m., and 2,3,6-TBA = 1,800 p.p.m. COPE (1966) noted delays in spawning of bluegill sunfish of up to two weeks after treatment of water with the propylene glycol butyl ether ester of 2,4-D at five and 10 p.p.m. However, no other effects were noted on reproduction or on survival of fry. In pond experiments, death of some fish as a result of 2,4-D treatment led to increased size in the survivors, probably as a result of the greater food supply available to the individual fish. In further trials with bluegill sunfish, HUGHES and DAVIS (1963) and DAVIS and HUGHES (1963) reported on effects of different formulations of 2,4-D and other auxins. Their tests showed 2,4-D and 2,4,5-T esters to have TLm_{24} ranging from 1.8 to 10 p.p.m. depending on the ester used. Dimethylamine salts of 2,4-D and 2,4,5-T had TLm_{24} of 162 to 542 p.p.m. and 144 p.p.m., respectively, compared to the alkyl amine salt of MCPA of 163.5 p.p.m. and of 2,4-D acid of 8.0 p.p.m. This work (which is referred to in COPE 1966, see above) shows the wide differences in toxicity that can occur in different formulations and the care which must therefore be taken in assessing the toxicity of an individual product before recommending it for use as an aquatic herbicide.

In addition to work on fish, WALKER (1962) has reported effects on a variety of bottom-feeding fish food organisms following application of 2,4-D to plastic enclosures at 1.0 to 4.0 p.p.m. LHOSTE (1959) has reported that ester formulations of 2,4-D or mixtures of 2,4-D and 2,4,5-T affected crustaceans, aquatic insects, and molluscs in the range of 0.1 to 3.3 p.p.m. In their investigations into the effect of the use of 2,4-D at rates from 40 to 100 pounds/acre in Tennessee Valley Authority reservoirs, SMITH and ISOM (1967) found no measurable toxic effect on benthic fauna or significant changes in mean numbers of burrowing mayflies (*Hexagenia*) before or after treatment. There were some deaths of caged fish but no dead or distressed free fish were found. Observations of free living fish indicated that they appeared to move out of the treated area, whilst analysis of a number of fish for 2,4-D residues gave no measurable results at the limit (0.14 p.p.m.) of detection. However, analysis for residues in mussels (mainly *Elliptio crassidens*) indicated that they concentrated the chemical from the surrounding water although no mortality was recorded. Analysis of mud samples showed that significant amounts of 2,4-D (up to 58.8 p.p.m. butoxyethanol ester) were present in isolated sediment samples up to 10 months after treatment. These fairly high levels in the environment of a sedentary animal such as the mussel might explain the residues found in these animals. RAWLS (1965) has reviewed literature

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on the toxicity of herbicides to estuarine fauna, principally crustacea and mollusca, and described experiments from his own laboratory. A variety of formulations of 2,4-D affected several species of animals at concentrations of one to 10 p.p.m. Larvae of oysters (*Crassostrea virginica*) were killed over a period of time by one p.p.m. of butoxyethanol ester and dimethyl salt of 2,4-D, and though oyster eggs were less susceptible, their development could be affected by 2,4-D butoxyethanol ester at 10 p.p.m. These effects might give some cause for alarm when rates of application of 20 to 30 pounds/acre are required for weed control in estuaries. Doses of this sort would give 7.34 to 11.02 p.p.m. if evenly distributed throughout one acre of water one foot deep. Because of tidal fluxes and uneven distribution only very local concentrations at toxic or near toxic amounts may occur in practice.

The results of these various investigations suggest that at herbicidal rates of application of auxins the hazards from acute or chronic toxicities to aquatic organisms are low. Nevertheless in some instances the dose rates required for effective herbicidal action, for example in estuaries or where the chemical is likely to be rapidly dispersed, may give rise to local and perhaps short term concentrations not far removed from those required for toxic effects on some organisms at susceptible stages of their life history.

VII. Wildlife in general

Hazards to wildlife from auxin herbicides have been reviewed by Rudd and Genelly (1956), Springer (1957), and Mellanby (1967). Whilst with any material having biological activity a risk of acute or chronic toxicity is always present, authenticated incidents of widescale poisoning of wild animals by these herbicides have not been reported. However, there is the further hazard to all forms of wildlife from herbicides of altering the habitat. Whilst herbicidal applications of the compounds to agricultural land undoubtedly modify the habitat there for wildlife, it is unlikely that individual instances of spray drift of the compounds at sublethal doses, although undesirable, have any really significant effect on habitat of adjoining non-cultivated land. This is not to say that some food plants for animals are not regularly killed in hedgerows and other field margins from spray drift, nor that in isolated instances of gross mishandling more severe effects do not occur over larger areas. It is probably true, although there is little or no scientific evidence, that the continued use of herbicides in the U.K. is gradually leading to the impoverishment of the flora of the field margins, and consequently to the gradual impoverishment of agricultural land as a whole as a habitat for wildlife.

The principal problem from the use of auxin herbicides in relation

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to wildlife, is not toxicological but ecological in terms of scale and intensity of use, especially on non-agricultural and industrial land. Thus the total destruction of a small area of vegetation on one occasion might be less serious for the wildlife of a region than the selective destruction of vegetation over a wide area at regular intervals. The hazard from the use of herbicides lies in the fact that they can now be used to affect vegetation over very wide areas in a short period of time, thus eliminating reservoirs of wildlife and wildlife habitat that would have otherwise survived.

Summary

Toxicities and hazards to man, domestic and laboratory animals, wildlife, bees, and other insects, soil animals and fish from MCPA, MCPB, 2,4-D, 2,4-DB, 2,4,5-T, mecoprop, and 2,3,6-TBA are discussed. In man only one authenticated instance of death by poisoning has been noted in West European and North American literature. Authenticated case histories of sublethal effects are also very rare. However, complaints of transient dizziness, sickness, and other symptoms are made from time to time by workers engaged in field applications, especially under conditions where the spray is inhaled excessively. The possibility of man acquiring toxic doses of these compounds in food, milk, or water appears to be very low.

Toxicity tests on a range of laboratory and domestic animals with a wide range of formulations of the compounds tend to show greater differences between formulations than between compounds. Amongst the domestic animals studied dogs appear to be more susceptible than the others. Acutely toxic doses of the compounds to farm animals and fowls are shown to be greater than the animals could normally ingest from treated vegetation or water following an agricultural application. Hazards to stock from the concentrated materials are emphasized, but the offensive and unpalatable nature of the undiluted chemicals would probably preclude any normal animal from ingesting a toxic dose. The danger of chronic toxicity is also shown to be very low and continuous high dosing over periods of months rather than weeks are required to produce severe symptoms of poisoning. In many animals a high proportion of the active chemical is voided in the urine within 24 to 48 hours of ingestion. Secondary hazards to animals as a result of changes in the chemical constituents of food plants may occur, for instance toxicity has been associated with temporary increases in the nitrate contents of some plants after spraying. Current recommendations for stock to be excluded from treated pasture for a period after treatment should be observed.

Hazards to wildlife exist from the use of herbicides but are also associated with other changes in agricultural practices. The effects of herbicides on habitat are serious for wildlife and should not be

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underestimated. Deaths of bees have occurred following spraying of nectar-producing plants when in flower. It is possible that similar hazards exist for other nectar-feeding insects. Where other insects have been studied, increases in population have been noted more often than decreases. With some aphids this has been associated with reduction in numbers of predators. No effects have been noted in studies of populations of soil arthropods. Increased activity by nematodes has been associated with the favourable conditions for invasion of the host plant created by auxin induced teratomas and other effects. Toxicity to fish and other aquatic organisms has been shown to depend on the formulation of the compound used. In general, toxicity to fish is low and herbicidal applications to water do not present a severe direct hazard. The dangers of secondary effects must be assessed in relation to other methods of control and the environmental characteristics of the water body to be treated. Some aquatic organisms other than fish may be directly affected at dose rates not very much in excess of those recommended for herbicidal use in water.

Résumé *

Sont discutés les toxicités et les dangers, pour l'homme, les animaux domestiques et de laboratoire, la vie sauvage, les abeilles et autres insectes, la faune du sol et les poissons, des composés suivants: MCPA; MCPB; 2.4.D.; 2.4.DB; 2.4.5.T; mecoprop et 2.3.6.TBA. Chez l'homme, un seul cas authentifié de mort par intoxication a été relevé dans la bibliographie de l'Europe occidentale et de l'Amérique du Nord. De même, les cas authentiques d'effets subléthaux sont très rares. Néanmoins, des sujets affectés à l'épandage de ces produits se plaignent, de temps à autre, particulièrement lorsque les conditions de dispersion exposent à une inhalation excessive, de vertiges passagers et d'autres symptômes et se sentent malades. L'éventualité d'absorption par l'homme de doses toxiques avec les aliments, le lait et l'eau semble très limitée.

Les épreuves de toxicité sur une grande variété d'animaux de laboratoire et d'animaux domestiques tendent à montrer que les différences sont beaucoup plus grandes en ce qui concerne les formulations qu'en ce qui concerne les matières actives. Parmi les animaux domestiques, le chien paraît être le plus sensible. Les doses susceptibles de provoquer des effets de toxicité aigüe chez les animaux de ferme et les volailles sont supérieures à celles que ces animaux pourraient ingérer à partir des plantes traitées ou de l'eau éventuellement polluée à la suite des applications en Agriculture. L'accent est mis sur les risques pour le bétail des formulations concentrées, mais la nature agressive et le mauvais goût de ces formulations rendent très impro-

* Traduit par R. Truhaut.

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bable l'ingestion d'une dose toxique par un animal normal. Les risques de toxicité chronique sont également très faibles, car des doses élevées doivent être répétées, non seulement pendant des semaines mais pendant des mois, pour pouvoir provoquer des symptômes graves d'empoisonnement. Chez beaucoup d'animaux, une forte proportion des matières actives est excrétée dans l'urine dans les 24 à 48 heures qui suivent l'ingestion. Des risques secondaires pour les animaux peuvent résulter de modifications dans les constituants chimiques des végétaux consommés; par exemple, des effets toxiques ont été associés à des augmentations temporaires des taux de nitrates de certaines plantes consécutives aux traitements. Il convient de respecter les recommandations relatives à l'exclusion du bétail des pâturages traités pendant une certaine période après le traitement.

Des dangers pour la vie sauvage peuvent résulter de l'usage des herbicides, mais ils peuvent également être causés par d'autres variations dans les pratiques agricoles. Les effets des herbicides sur l'habitat sont graves pour la vie sauvage et ne doivent pas être sous-estimés. Des morts se sont produites chez les abeilles à la suite de traitements de plantes mellifères à l'époque de la floraison. Il est possible que des risques analogues existent pour les insectes qui se nourrissent du nectar des fleurs. Les études relatives à d'autres insectes ont révélé des augmentations de populations plus fréquemment que des diminutions. Dans le cas de quelques espèces de pucerons, cet accroissement était associé à une réduction du nombre des prédateurs. Aucun effet n'a été observé dans les études sur les populations d'arthropodes du sol. L'accroissement d'activité des nématodes était lié aux conditions favorables pour l'invasion des plantes hôtes créées par les tératomes et autres effets dûs aux auxines. La toxicité pour les poissons et les autres organismes aquatiques s'est révelée dépendre du type de formulation utilisée pour l'épandage de la matière active. En général, la toxicité pour les poissons est basse et les applications d'herbicides à l'eau ne présentent pas de risques directs graves. Les risques d'effets secondaires doivent être évalués en liaison avec d'autres méthodes de lutte et les caractères de l'environnement des eaux à traiter. Quelques organismes aquatiques autres que les poissons peuvent être directement affectés à des doses ne dépassant pas beaucoup celles recommandées pour l'emploi des herbicides dans les eaux.

Zusammenfassung *

Toxizität und Gefährdung bei Mensch, Haus- und Laboratoriumstieren, Wild, Bienen und anderen Insekten. Bodentieren und Fischen durch MCPA, MCPB, 2,4-D, 2,4-DB, 2,4,5-T, Mecoprop und 2,3,6-TBA

* Übersetzt von F. Bär.

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werden diskutiert. Beim Menschen wurde nur ein verbürgtes Beispiel eines Todesfalles infolge Vergiftung in der westeuropäischen und nordamerikanischen Literatur berichtet. Verbürgte Fälle von sublethalen Effekten sind auch sehr selten. Jedoch werden von Zeit zu Zeit Klagen über vorübergehendes Schwindelgefühl, Übelkeit und andere Symptome von Arbeitern vorgebracht, die in der Anwendung auf dem Felde tätig sind, besonders unter Bedingungen übermässiger Spray-Inhalation. Die Möglichkeit für den Menschen, toxische Dosen dieser Verbindungen in Lebensmitteln, Milch oder Wasser zu erreichen, scheint sehr gering zu sein.

Toxizitätstests bei einer Reihe von Laboratoriums- und Haustieren mit einem weiten Bereich von Formulierungen der Verbindungen neigen dazu, grössere Differenzen innerhalb der Formulierungen als zwischen den Verbindungen zu zeigen. Von den untersuchten Haustieren scheinen Hunde empfindlicher zu sein als andere. Die akuttoxischen Dosen der Verbindungen für Vieh und Geflügel waren höher als sie die Tiere normalerweise aus behandelten Pflanzen oder aus Wasser nach Anwendung in der Landwirtschaft aufnehmen können. Die Gefährdung des Viehbestandes durch konzentriertes Material wird hervorgehoben, aber die agressive und widerwärtige Natur der unverdünnten Chemikalien würde wahrscheinlich jedes normale Tier von der Aufnahme einer toxischen Dosis abhalten. Die Gefahr der chronischen Toxizität erwies sich auch als sehr gering und eine kontinuierliche hohe Dosierung eher über Monate als über Wochen ist erforderlich, um schwere Vergiftungssymptome zu erzeugen. Bei vielen Tieren wird ein hoher Anteil der aktiven chemischen Stoffe im Harn 24 - 48 Stunden nach der Aufnahme entleert. Sekundäre Gefährdung bei Tieren als Ergebnis von Veränderungen der chemischen Bestandteile von Nahrungspflanzen kann vorkommen; zum Beispiel war die Toxizität begleitet von einem vorübergehenden Anstieg des Nitratgehaltes gewisser Pflanzen nach dem Besprühen. Laufende Empfehlungen für die Fernhaltung des Viehbestandes von der behandelten Weide für einige Zeit nach der Behandlung sollten befolgt werden.

Eine Gefährdung für Wild besteht durch die Anwendung von Herbiziden; sie ist jedoch ebenso verbunden mit anderen Änderungen in der Landwirtschaftspraxis. Die Effekte der Herbizide auf dem Standort beim Wild sind ernst und sollten nicht unterschätzt werden. Tod von Bienen erfolgte nach dem Besprühen von Nektar-erzeugenden Pflanzen in der Blüte. Es ist möglich, dass eine ähnliche Gefährdung für Nektar-fressende Insekten besteht. Beim Studium anderer Insekten wurde ein Populationsanstieg häufiger als eine -abnahme festgestellt. Bei Blattläusen war dies verbunden mit der Herabsetzung der Anzahl der Raubinsekten. Keine Effekte wurden festgestellt in Untersuchungen von Populationen von Bodenarthropoden. Eine gesteigerte Aktivität durch Nematoden war verbunden mit günstigen Bedingungen für den Befall der Wirtspflanze, erzeugt durch Auxin-induzierte Teratome und

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andere Effekte. Die Toxizität für Fische und andere Wasserorganismen zeigte eine Abhängigkeit von der Formulierung der angewandten Verbindung. Im allgemeinen ist die Toxizität für Fische gering und die Herbizidanwendung zum Wasser stellt keine schwere direkte Gefährdung dar. Die Gefahren sekundärer Effekte sind in Beziehung zu setzen zu anderen Kontrollmethoden und zu den Umwelteigenschaften der zu behandelnden Wasserfläche. Ausser den Fischen können gewisse Wasserorganismen direkt beeinflusst werden bei Dosisbeträgen, die nicht sehr weit über den zur Herbizidanwendung im Wasser empfohlenen liegen.

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Reptile 2,4-D Toxicology
from Health Aspects of Pesticides
Abstract Bulletin Jan 1970 p. 1-12

c.c. M.A. Wolf Bircher
Ken Holder Medical
from M. Ling 3/9/70

70-0152. Zink, P. (Institut fuer Gerichtliche Medizin und Kriminalistik, Universitaet Erlangen-Nuernberg, Universitaetsstrasse 22, 8520 Erlangen, Germany). Identification of various herbicidal chlorinated phenoxycarboxylic acids by means of mass spectrography. *Arch. Toxikol. 25(1): 1-4; 1969.* (5 references) (German)

Two cases of poisoning described in the literature (Geldmacher-von Mallinckrodt and Lautenbach, this journal, 21: 261; 1966) served as the stimulus to assess the possibilities of identifying chlorinated phenoxycarboxylic acids by mass spectrography since the presence of very small quantities of the substances made analysis difficult. Comparative spectrograms were prepared, using the CH 4 (Atlas-Werke MAT) mass spectrometer, for MCPA (2-methyl-4-chlorophenoxyacetic acid), MCPB (*gamma*-(4-chloro-2-methylphenoxy) butyric acid, MCPP (*alpha*-(4-chloro-2-methylphenoxy)-propionic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4-DP (*alpha*-(2,4-dichlorophenoxy)-propionic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). The highest peak values are tabulated in mass units and on the basis of 100%. The number of carbon atoms in each compound was another differentiating characteristic and this could be calculated for all the compounds from the molecular peak groups, which were high for the mass spectra of all the substances. In the two cases of intoxication cited above, liver and urine were the specimens analyzed. A crystalline substance was obtained from the urine extract (acidic medium with ether and subsequent vacuum sublimation in temperature gradients after Schmidt's method) which precipitated four insignificantly separated fractions in the sublimation tube on the sublimation layer. The fractions analyzed into a mixture of 2,4-D, MCPA and MCPB, a little 2,4,5-T and traces of 2,4-DP; each fraction showed the same mixture relationships. The liver extract (after Valov) yielded a greasy residue. Mass spectrographically this proved to be contaminated with a chlorine-free compound. Small amounts of 2,4-D were identified by the molecular peak group at 220 with the typical 2-chloro distribution as well as peak groups of the main fractional part at 162; other chlorinated carbonic acids were not found. Thus, the analysis showed the compound to be pure 2,4-D.

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and I realized then that attending this meeting was most valuable in showing what the beekeeper is up against in trying to preserve his bees from indiscriminate application of insecticides.

The only salvation for the beekeeping industry is for each and every beekeeper to keep hammering home, especially to our city fathers, the importance of the honeybee and the deva-

Beech he asked me what I would p
er have, a few dead bees or an
demic of encephalitis and I asked
in turn what constituted an epide
and his parting remark was "one ca

I thought to myself as I watched
plane leave the ground that poss
many of these expensive progr
have been sold on fear, and fear c
rather than on their actual need.

Safeguarding the Honeybee

MN 073280

From N. J. Beekeepers' Assn. News

IN COOPERATIVE studies with the Arizona Agricultural Experiment Station, Tucson, plant physiologist Howard L. Morton and entomologists Joseph O. Moffett, and Robert H. Macdonald, all of ARS, tested the toxicity of 15 herbicides on honeybees under controlled laboratory conditions.

They fed herbicides at concentrations of 0, 10, 100 and 1,000 parts per million (ppm) in a 60-per cent solution of sucrose syrup to newly emerged honeybees. These levels are much higher than normally used to control weed pests in crops. The results were:

Non-toxic at all concentrations. Simazine, dicamba, 2,4-D, picloram, 2,4,5-T, amiben, 2-chloroethanephosphonic acid, and 2,3,6-TBA.

Extremely toxic at 100 and 1,000 ppm: Paraquat, MAA, MSMA, DSMA, hexafluarate, cacodylic acid, and e
thali.

Moderately toxic at 10 ppm: P
quat, MAA, MSMA, DSMA, and
afluarate.

Amine salts of 2,4-D and 2,4,
ester of 2,4,5-T, and potassium sal
picloram applied in water did not
crease bee mortality. But an ester
2,4,5-T and an ester of silvex app
in a diesel oil carrier caused high m
tality on the first day after treatm
All bees sprayed with paraquat, ca
dylic acid, and MSMA in water
within 12 days.

While the results of the experime
are encouraging, further studies,
cluding field tests, are needed to de
mine wether the herbicides
proved toxic to honeybees in these
periments are hazardous at rates
quired for weed control.

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The Analysis of 2,4-D Acid for 2,3,7,8-Tetrachlorodibenzo-p-dioxin Content

Dow Lot No.	PPM Dioxin Run 1	Run 2	Run 3
091500	<1	only enough sample for one run	
091530	<1	<1	<1
091630	<1	<1	<1
091640	--	<1	<1

Recovery studies indicate about 70% at the 2 and 4 part per million level. The above results are based on the difference in response between a spiked and unspiked sample at the 1 ppm level. Peak area was used in the calculations.

W.B.C. 4/13/70

C0005736

DIOXINS ASSAY IN 2,4-D and SILVEX

The analytical method for 2,3,7,8-tetrachlorodibenzo-p-dioxins does not work with 2,4-D or silvex. However, we have bioassayed these compounds for chloracne response in the rabbit ear test and found them negative. (< 1 ppm of TCDBD) See attached letter.

DOW 979351

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CROP RIDER AMINE 2,4,5-T

Diamond Alkali Company

Contains 4 lb 2,4,5-T/gallon

Triethylamine salt of 2,4,5-T 57%

DOW 0793352

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THE DOW CHEMICAL COMPANY

MIDLAND
April 13, 1970

G. E. Lynn
Ag. Products
9008 Building

DOW 979353

RESULTS OF CHLORACNE STUDIES CONDUCTED ON ASSORTED 2,4-D, 2,4,5-T, SILVEX SAMPLES AND THEIR ESTERS, ORANGE AND DIAMOND'S CROP RIDER AMINE 4T-2.

As per our telephone conversation on April 1, 1970 here is a summary of results of all chloracne studies recently completed on assorted 2,4-D, 2,4,5-T, Silvex Samples and their Esters, Orange and Diamond's Crop Rider Amine 4T-2.

Chloracne T-Number	Name	Reference Number	Chloracne Response
2578	2,4,5-T	120449	Negative
2586	2,4,5-T	120349	Negative
2587	2,4,5-T	120369	Negative
2588	2,4,5-T	120419	Negative
2589	2,4,5-T	120459	Negative
2590	2,4,5-T	120479	Negative
2601	2,4,5-T	120070	Negative
2609	2,4,5-T	Butyl Ester 60/40 PPI-93-3	Negative
2611	2,4,5-T	Iso Octyl Ester PPI-93-5	Negative
2612	2,4,5-T	Dowanol Ester (PiB) PPI-93-6 Lot # 130667	Negative
2602	2,4-D	Lot # 90530	Negative
2603	2,4-D	Lot # 90540	Negative
2604	2,4-D	Lot # 90670	Negative
2605	2,4-D	Lot # 90970	Negative
2606	2,4-D	Lot # 94847	Negative
2613	2,4-D	Butyl Ester 60/40 ASI	Negative
2669	2,4-D	Iso Octyl Ester AS-7 2/5/70	Negative
2670	2,4-D	Iso Octyl Ester DE 20 A 2/6/70	Negative

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90405d. Synthesis of dihydrojasmone, a component of tea essent oil (Sect. 30) 91073b. Changes in chem. compn. of molas depending on period of year in which sugar beets were proces

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18—PLANT-GR

90143s Toxicity and hazards to man, domestic animals, a wildlife from some commonly used auxin herbicides. Wa John Michael (Monks Wood Exp. Sta., Huntingdon, Engl *Residue Rev.* 1969, 26, 37–62 (Eng). Auxin-type herbicides are reviewed, with some possibly new interpretations. Repo of toxicity of the I in man mainly refer to accidental poisoning children. It seems probable that toxic hazards from residues I in foods are very small. Acute and chronic toxicities report in animals have been based on amts. of I well in excess of t amts. likely to be eaten as pasturage or other food. Howev it is possible that application of I to nitrate-accumulating plan increases the hazard of nitrate poisoning in herbivorous anima Applications of I to plants in flower can cause a real hazard to be (*Apis mellifera*) and possibly other nectar-feeding insects. F soil animals and fishes the hazards of I are low, but vary wi the formulation applied. Only 1 authenticated fatality in m from the action of I has so far been reported. 102 references. W. C. Tobie

90144t Herbicides and soil biology. Gorzelak, Andrz *Sylwan* 1969, 113(3), 43–8 (Pol). A review indicates that a n of herbicides used in weed control, including triazines, chlorinat fatty acids and phenoxyacetic acids, and chlorates, cause d turbances in soil microorganisms, esp. in forests which are lea disturbed by human activities. Although herbicides were al inhibitory to soil organisms under conditions of cultivation, it w concluded that the effects were negligible and completely d appeared during the year following application.[5648] J. Klenha

90145u Binding of salts and esters of 2,4-D, 4-chloro-methylphenoxyacetic acid and 2,4,5-T in soil and natural sorbent

levels administered, did not result in signs of cholinesterase inhibition. Carbaryl administered daily at 300 mg/kg to the guinea pig from day 11 to 20 produced a maternal mortality of 38% and fetal mortality of 17.5%, but no terata were observed. After the fetuses were cleared and stained, examination of the skeleton disclosed bone defects appearing mostly in the cervical vertebrae. When carbaryl administration was repeated as a single treatment on selected days between day 11 and 20, terata were found only in some of the litters treated on days 12 and 16 of gestation. The teratogenicity of DMSO in the hamster at 1 mg/100 g body weight was confirmed. Thiram was teratogenic when suspended in CMC at 250 mg/kg; when it was dissolved in DMSO the teratogenicity was additive or possibly more than additive. When DMSO was used as a solvent for disulfiram, the high levels of disulfiram were more teratogenic than was DMSO alone. Disulfiram as a CMC suspension was not teratogenic. Ref: Health Aspects of Pesticides, Vol. 3, No. 5, May, 1970.

2,4,5-T CANCELLATIONS - U.S.A

In mid-April the Pesticide Regulation Division of USDA suspended the use of all formulations of 2,4,5-T for uses in lakes, ponds or on ditch banks and suspended liquid formulations for use around the home, recreation areas, and similar sites.

On May 1 cancellation of all granular formulations for use around the home, recreation areas, and similar sites, and all uses of the chemical on food crops intended for human consumption was announced. Cancellation or suspension was based on new research information which show 2,4,5-T to cause abnormal development in unborn mice. The Surgeon General of the Department of Health, Education and Welfare says that exposure to this herbicide may present an imminent hazard to women of child bearing age. These actions do not eliminate registered use of 2,4,5-T for control of weeds and brush on range, pasture and forests or on right-of-ways and other non-agricultural land.

No cancellation of uses have beenannounced for 2,4-D or 2,4,5-TP(silvex) which are used in many lawn formulations. Ref: Chemicals Pesticides Program, Vol. 6, No. 2, May 13, 1970.

DDT MAY INTERFERE WITH PAIN MEDICATION, ACCORDING TO RODALE'S HEALTH BULLETIN.

The discovery was made by scientists of the Karolinck Institute of Sweden who found that the action of antipyrine, a fever-pain medication, was interfered with by pesticides. According to the researchers, the amount of antipyrines in the blood decreased by half in about seven hours in those who were exposed to DDT and other pesticides, while the normal half-life of the anti-pain medication was about thirteen hours. It is believed that insecticides stimulate production of enzymes in the liver which metabolize the drug more quickly. Ref: Environment Jan./Feb. 1970.

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Epidemiology, Prevention and Treatment

d by organophosphorus insecticides and to reverse
of acetylcholinesterase. Atropine protects the
ein against acetylcholine, but it does not reverse
on of acetylcholinesterase. Animals injected with
doses of organophosphorus insecticides have
r treatment with PAM in combination with
mba and Hiraki (1958), Wilford (1970) and an
cf. *News 198: 137: 1970)* support the use of
injunction with atropine as an antidote against
ate insecticide poisoning.

i, J. S.; Lee, H. H.; Kim, H. Y.; Hong, W. P.; Lee,
of Internal Medicine, College of Medicine, Pusan
Pusan, Korea). **Clinical observations on parathion
in 52 cases.** *Taehan Naekwa Hakkoe Chapchi
1970.* (17 references) (Korean)

observations were made in 52 cases of acute
oxication studied in the Department of Internal
an University Hospital from January 1965 to
The following results were obtained. The causes
ing were: 1) suicidal attempt (69%), 2) intoxica-
aying (38%) and 3) one case of intoxication due
g and one due to ingestion of contaminated
ales were affected three times more often than
ersus 25%). The peak incidence of poisoning in
patients was between the ages of 20 and 39. In
onal prevalence, intoxication was most common
r, especially in July and August. Doses of 6 to 21
e were required for full atropinization (1½ to 8
doses of 10 to 58.5 mg for clinical recovery (8 to
opine and 2-PAM were applied simultaneously in
except one recovered clinically. Therapeutically,
ry from parathion intoxication was achieved in
%) and 13 cases (38%) died. (Author abstract

z, E.; Nass, H. (Klinik fur Tiergeburtshilfe und
nkheiten, Freie Universitaet, Koenigsweg 65, 1
rmany). **E 605 poisoning in a herd of Berlin
rtl. Umschau 25(3). 128-31. 1970.** (16
rman)

lent of poisoning following the use of E 605
a skin spray to control mange in a group of 19
d two bulls is described. The toxicant as a 0.25%
sprayed in such a way that the heads of the
mangers of foodracks were exposed. The dosage
4 mg parathion/kg body weight. Of the 21
were affected. In the two bulls, symptoms
n the day of exposure. One bull after 10 days of
wered; the other died after 4 days of treatment.
at was last to develop symptoms did so on the
xposure day. The rest of the cattle displayed
ssuming variously on postexposure days 1, 2, 3,
e symptoms observed were: massive salivation,
sea, muscle tremors, groaning, noisy out-
eakness of the hindquarters and partial
nd refusal of feed. Body temperature and pulse
ormal range. Of the 17 affected animals, five
died. Treatment consisted of i.v. atropine (50 to 200 mg/animal) and Toxogonin-Merck (1500 to 1750 mg/animal). This was accompanied by symptomatic treatment. To counteract great fluid loss, infusion of glucose and electrolytes were given to a total of 54 l/animal. To counteract diarrhea, medication by nasal intubation was applied. Other drugs were given to sustain the blood system and the liver. With this treatment 12 animals (11 cows and one bull) survived. Milk production drastically declined. Analysis of the brain of some of the dead animals showed cholinesterase activity to be at the lower limit of detectability. In the urine of two animals, *p*-nitrophenol, a metabolite of E 605, was observed. The detailed symptoms of the illness, duration of treatment and outcome in each of the 17 affected animals are tabulated. Four animals recovered without treatment. A general discussion of the nature of organophosphorus intoxication and of the relation between the anticholinesterase activity of these compounds and/or their breakdown products and the pathogenic process and its symptoms is presented.

K-3712 K-4581

71-0709. Pinsent, P. J. N.; Lane, J. G. (Dept. of Veterinary Medicine, U. of Bristol, Langford House, Langford, Bristol, England). **A case of possible 2,4-D and 2,4,5-T poisoning in the horse.** *Vet. Record 87(8): 247. 1970.*

The possibility that the ingestion of nettles and herbage that had been treated or contaminated with a mixture of 2,4-D and 2,4,5-T was responsible for the illness and death of several horses is considered. A mixture containing the above herbicides was used to destroy nettles around the edge of a pasture containing six horses. The wind carried the spray into the grazing area contaminating the water trough and a considerable quantity of the herbage. Four of the horses were removed from the pasture after 24 hr. The two other horses remained for 12 days. They were both brought in when one of them became ill. Early symptoms included depression, disinterest in drinking water, salivation, smacking of lips and jaundice of the mucous membranes. Alimentary stasis developed with some abdominal distension and pain. Except for small amounts of dark feces adhering to the mucous membrane, the rectum was empty. No feces were passed. The symptoms also included muscular tremors, patchy sweating, regurgitating of foul-smelling stomach contents and the inability to swallow. Water which was taken in after the animal developed a thirst ran back out of the mouth. Body temperature was normal but the pulse increased to 80/min. The animal was destroyed after 10 days of illness. Red and white cells were within the normal range. SGOT values had increased to 157 mu/ml, while blood urea increased to 300 mg/100 ml. There were 100 mg of protein/100 ml of urine. The second horse developed similar symptoms and died within 24 hr after removal from the pasture. Autopsy findings included extensive lesions in the esophagus and stomach with degenerative changes in the liver and kidneys. Histological examination revealed a proliferation of squamous epithelium of the esophagus and stomach with some interspersed ulcerated areas. There was severe fatty change in the liver and some evidence of glomerular and renal tubular damage. Two other horses became ill but survived. At this writing, the exact cause of the illness and deaths is undetermined.

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70-0344. Galston, A. W. (Dept. of Biology, Yale U., New Haven, Conn. 06520). Plants, people and politics. *BioScience 20(7): 405-10; 1970.* (17 references)

Contemporary man is confronted with many problems concerning social unrest, pollution and overpopulation. Some of the chemicals that man depends so much on to produce high levels of agricultural products are having deleterious effects on man and his environment. Chlorinated hydrocarbons like DDT, dieldrin and aldrin, once considered very useful, have now become menaces because of their persistence in the biosphere and their potential poisoning of various kinds of creatures. Present and future pesticides must be more thoroughly tested and then chosen and used with greater selectivity. Some of the more recently introduced herbicides have been shown to be potential herbicidal analogs of DDT. One of the suspected chemicals is 4-amino-3,5,6-trichloropicolinic acid (picloram or Tordon). Under optimum conditions in some soils, 20 to 50% of applied picloram disappeared after 467 days (Youngson *et al.*, 1967). On other soils low in moisture and poor in inorganic matter and air, only 3.3% of the applied picloram disappeared in a similar period. It takes between 10,000 and 100,000 parts of exogenous carbon to oxidize one part of Tordon (Tschirley, 1969). The dangers of further use of this herbicide are evident. Another chemical, 2,4-D, can also become a menace under certain conditions by causing a massive increase in the nitrate content of pasture plants as to sicken animals eating these plants (Stabler and Whitehead, 1950) and must be used very carefully. Pesticides fabricated around heavy metals, such as lead and mercury, or other elements such as arsenic (trivalent) do not become completely detoxified once applied. The presence of rising quantities of lead and mercury in the

environment has caused growing concern in public health. Science must not be misapplied. Thoughtless and indiscriminate use of chemicals such as 2,4-D, picloram, 2,4,5-T and arsenical cacodylic acid by industry, the military and other groups and organizations has and may continue to cause ecological havoc. The legal structure of our government as well as the influence of international committees and commissions offer many ways to promote rational use and limited control of our environment and must be employed before it is too late.

70-0845. Anonymous. Agriculture will continue to approve 2,4,5-T registration. *Chem. Eng. News 48(16): 21; 1970.*

Despite a first round of Senate hearings, Agriculture will continue to register uses for 2,4,5-T, with restrictions, if any, depending on the results of tests and research currently under way. Ned Bayley, Director of Science and Education for Agriculture, holds that there is insufficient evidence to suspend or cancel registration. In reference to the study by Bionetics Research Laboratories showing that 2,4,5-T induced birth defects in laboratory animals, it was stated that "preliminary" government studies indicated that the defects were due to contaminant dioxins rather than to 2,4,5-T itself.

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BCM had been formed. Similar experiments with thiophanate yielded BCM. The findings indicated that the fungitoxicity of methyl thiophanate and thiophanate may come from their transformation into the more potent fungicides, BCM and the ethyl analog of BCM, respectively.

71-1433. Bahr, T. B.; Ball, R. C. (Inst. of Water Res., Michigan State U., East Lansing, Mich. 48823). Action of DDT on evoked and spontaneous activity from the rainbow trout lateral line nerve. *Comp. Biochem. Physiol. A 38(2): 279-84; 1971.* (8 references)

The possibility that DDT-induced tremors and convulsions in rainbow trout (*Salmo gairdneri*) could arise from postsynaptic events initiated by intense afferent activity impinging on the central neurons was investigated by monitoring the frequency of spontaneous discharge and the patterns of evoked discharge from the lateral line nerve before and after the onset of tremoring. The trout were anesthetized with 100 mg MS 222/l and permanently immobilized by severing the spinal cord at the foramen magnum. The fish were placed on their sides in a water-filled chamber (13 C) and their gills were irrigated. Bipolar silver wire electrodes were looped under the distal end of the centrally cut lateral line nerve midway along the trunk and water was excluded from the recording site. Neural activity was monophasically displayed on a Tektronix 502A cathode ray oscilloscope after amplification (Grass P-8). The DDT dissolved in ethanol was administered in the water (15 ppm for 6 hr prior to preparation) or directly into the blood by cannula (0.01, 0.1, 0.2, 0.4, 0.5, 1.0 and 2.0 mg DDT dissolved in ethanol and suspended in Courtland's saline). Responses were evoked by directing drops of water to the surface of the water over the innervated portion of the lateral line. The frequency of spontaneous neural discharge, as determined by electrically counting the compound action potentials on a decade scale and from photographs of oscilloscope displays, revealed no apparent differences between treated and untreated fish with either method of DDT administration. Since obvious tremoring in the absence of lateral line involvement was observed, it is believed that afferent activity from the lateral line nerve does not play an important role in nor provide a sensitive index for analyzing the neurotoxic action of DDT but that the central nervous system is the primary target for DDT in fish.

71-1434. Lutz-Ostertag, Y.; Lutz, H. (Laboratoire de Biologie Animale, Faculte des Sciences, Complexe Scientifique des Cezaux, B.P. No. 45, 63-Aubiere, Puy-de-Dome, France). Deleterious effect of the herbicide, 2,4-D, on the embryonic development and fecundity of winged game. *Compt. Rend.; Ser. D 271(25): 2418-21; 1970.* (French)

The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) was studied on the development of the embryos of the pheasant (*Phasianus colchicus*), red-legged partridge (*Alectoris rufa*) and grey partridge (*Perdrix perdrix*). Nonincubated eggs and eggs incubated for 3½ days were placed in artificial nests and sprayed with a 2,4-D solution which was applied at a rate of 1 and 2 l/ha. The treated eggs were aired and turned twice daily. Control eggs were sprayed with pure water. The embryos were sacrificed between the 20th and 22nd days of development. Eggs of the three species used usually hatch after about 24 days of incubation. 2,4-D was very toxic to the developing embryos. Of the 520 pheasant eggs, 345 red-legged partridge eggs and 201 grey partridge eggs, 399, 148 and 148 embryos, respectively, died prior to the 19th day of incubation. Most of the surviving chicks were either partially or completely paralyzed. The embryos from the treated eggs were smaller than the normal control embryos of the same age. They exhibited a series of anomalies including lordosis of the neck, fusion of certain cervical vertebrae, atrophy of the leg muscles and clenched digits of the hind limbs. Feathers of the ventral regions were depigmented and certain pterylae were absent. The chicks were so deformed that they could not break out of the eggshell at hatching. 2,4-D also had pronounced effects on sexual differentiation during the development of the embryos of all three species. The test compound seriously affected the fertility of the treated birds.

71-1435. Bratkowski, T. A. (The U. of Wisconsin, Madison, Wis. 53706). Isolation and properties of a DDT-sensitive adenosine triphosphatase complex from rat brain. *Dissertation Abstr. Intern. 31(9): 5149B-50B; 1971.*

Copies of the thesis are available from University Microfilms, order No. 70-22,637.

71-1436. Roelofs, R. D. (Oregon State U., Corvallis, Oreg. 97331). Effects of dieldrin on the intrinsic rate of increase of the guppy, *Poecilia reticulata* Peters. *Dissertation Abstr. Intern. 31(9): 5319B-20B; 1971.*

Copies of the thesis are available from University Microfilms, order No. 71-6785.

71-1437. Kapoor, I. P. (U. of Illinois at Urbana-Champaign, Urbana, Ill. 61801). Comparative metabolism of DDT, methoxychlor and methiochlor in mammals, insects and in a model ecosystem. *Dissertation Abstr. Intern. 31(9): 5408B-9B; 1971.*

Copies of the thesis are available from University Microfilms, order No. 71-5145.

71-1438. Kuhn, P. J. (U. of Massachusetts, Amherst, Mass. 01002). Microbial interaction with diazinon, an organophosphate pesticide. *Dissertation Abstr. Intern. 31(9): 5511B; 1971.*

Copies of the thesis are available from University Microfilms, order No. 71-6388.

71-1439. Benz, F. W. (The U. of Iowa, Iowa City, Ia. 52240). Stereo- and bio-chemical evaluation of a series of oxime reactivators of diethylphosphorylacetylcholinesterase. *Dissertation Abstr. Intern. 31(9): 5526B-7B; 1971.*

Copies of the thesis are available from University Microfilms, order No. 71-5712.

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NB T25.14-293-4

CHEMICAL BIOLOGY RESEARCH — DOW CHEMICAL U.S.A. — FILE NB T23.14-11-14, NB T25.14-97-5

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G. E. Lynn	199-0000909 177-9007000	8/24/71	2372, 20054, 7797

THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) AND ESTERS OF 2,4-D ON RAT EMBRYONAL, FETAL AND NEONATAL GROWTH AND DEVELOPMENT

BAS P. J. Gehring gls

REPORTED BY: B.A.Schwetz, G.L.Sparschu CHECKED BY: P. J. Gehring PJG

INFORMATIVE SUMMARY WITH CONCLUSIONS BASED ON THE SAMPLE RECEIVED. ADDITIONAL INFORMATION INCLUDING THE EFFECT REPEATED EXPOSURE MAY BE REQUIRED AS SPECIFIC USES AND FORMULATIONS ARE DEVELOPED OR IF PROCESS CHANGES OCCUR

This study evaluated the effects of 2,4-D, the propylene glycol butyl ether ester of 2,4-D (PGBE) and the isooctyl ester of 2,4-D (IO) on fetal development and neonatal growth and survival. Dose levels of 2,4-D up to a maximum tolerated dose of 87.5 mg/kg/day or molar equivalents of PGBE or IO were administered to pregnant Sprague-Dawley rats on day 6 through 15 of gestation. Fetuses were delivered by Cesarean section on day 20 of gestation and were examined grossly, measured and weighed. Following routine preparations, the soft tissues and skeletons were examined. Signs of embryotoxicity and fetotoxicity, such as decreased fetal body weight, subcutaneous edema, delayed ossification of bone, lumbar ribs and wavy ribs were observed at high dose levels; considered overall, the responses were dose related. Teratogenic responses, however, were not seen at any dose level. 2,4-D did not affect fertility, gestation, viability or lactation. PGBE and IO had no effect on fertility and gestation indices, but highest dose levels decreased viability and lactation indices. Neonatal growth and development were not altered by treatment during pregnancy.

DOW257241 MN022777

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72

Japanese Journal of Industrial Health
Vol. 12, Number 9 (October, 1970)

THE FATE OF 2, 4, 5-TRICHLOROPHENOXYACETIC ACID IN MAN

Akihito MATSUMURA†

2, 4, 5-トリクロロフェノキシ酢酸の人体内における運命

松村昭仁†

The effectiveness of chlorinated phenoxyacetic acids as plant growth regulators has been recognized for a number of years. In earlier reports[1-3], 2, 4-dichlorophenoxyacetic acid (2, 4-D) was noticed to be non-toxic to animals and man. But later, the toxicity of 2, 4-D and 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) for experimental animals has been revealed by various papers[4-6].

Although tremendous amounts of 2, 4-D, 2, 4, 5-T and the related compounds have been manufactured and used, clinical reports of poisoning are rare. Nielsen[7] reported a case of suicide with ingestion of diethylamine salt of 2, 4-D. He observed 6.0 g of 2, 4-D in the corpse of the victim, corresponding to 30 mg/kg. Seabury[8] reported another case. He administered to a patient, suffering from disseminated coccidioid mycosis, 3.6 g of sodium salt of 2, 4-D through intravenous infusion. The patient was troubled with twichings of the muscles and fell into stupor, but recovered.

A couple of years ago, the author found such symptoms and signs as nausea, vomiting, weight loss, headache, fatigue and skin rash among the workers of the test operation mixing an ammonium-sulfamate derivative, 2, 4, 5-T and two other kinds of chemicals. Though the epidemiological study revealed 2, 4, 5-T to be noxious, the author failed to support the finding because the fate of 2, 4, 5-T in the human body was obscure in those days.

Unsubstituted or p-chlorophenoxyacetic acids are excreted in urine by man, dog and rabbit in an intact form because of the ether bondage[9-10]. Clark[11], using C^{14}-labelled 2, 4-D, demonstrated that 95% of orally administered 2, 4-D was excreted unchanged by sheep within 72 hours. Leigh[12] et al. reported the fate of 2, 4, 5-T in dairy cows. Out of 454 mg fed, 430.7 mg of the 2, 4, 5-T was recovered from the urine. These facts suggest that analogically in the case of man, orally administered 2, 4, 5-T will be excreted in the urine. The author examined whether or not ingested 2, 4, 5-T was excreted in the urine unchanged and investigated the fate of it from the pharmacodynamic point of view.

Subjects

A volunteer (23-year-old male) was ingested with 150 mg (2.2 mg/kg) of 2, 4, 5-T after breakfast. Blood samples were collected at 1-to 2-hour intervals during the first 6 hours and less frequently thereafter.

Two volunteers (27- and 23-year-old males) were given 100 mg of 2, 4, 5-T orally. Urine samples were collected at 3- to 6-hour intervals for 72 hours.

Collections of 24-hour urine were obtained from 21 workers of a chemical manure factory.

Methods

1) Determination of 2, 4, 5-T

Determination of 2, 4, 5-T was carried out by Nielsen's method with modifications as follows.

Twenty milliliters of diluted urine (two- or three-fold) or diluted blood plasma (three- or fourfold) was taken into a 50 ml-centrifuging tube with a glass stopper. After adding 1 g of crystalline tartaric acid, 4 g of anhydrous Na_2SO_4, 3 ml of chloroform and 10 ml of ethyl ether, the tube was vigorously shaken for 5 minutes and centrifuged for 3 minutes at 1,200 G. The organic phase was decanted and extraction of the residue was performed twice more with 3 ml of ether. The combined organic phase was extracted twice with 15 ml and 3 ml of 1/30 M phosphate buffer (pH 7.4). After being washed with half the volume of chloroform, the combined phosphate buffer extract was rendered strongly acid with 1.5 ml of 3N-H_2SO_4 to be extracted twice with chloroform. The chloroform solution was extracted with 20 ml of borate buffer (pH 10.6). The aqueous solution was extracted

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with 20.0 ml of chloroform after it had been made strongly acid with 1.5 ml of 3N-H_2SO_4. To remove the turbidity, the organic extract was filtrated through anhydrous Na_2SO_4.

One milliliter or two (or more, in the case of low concentrations) of the chloroform solution was poured into a small flask to make it evaporate cautiously just to dryness. Two milliliters of chromotropic acid in conc. H_2SO_4 solution was poured into the flask, which was prepared by dissolving 0.10 g of the sodium salt of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) in 100 ml of concentrated sulfuric acid. Ten milliliters (or more according to the concentrations) of $SnCl_2$ solution prepared by dissolving 25 g of stannous chloride, $SnCl_2 \cdot 2H_2O$, in 500 ml of 2N-H_2SO_4 was added to the flask after it had been heated at 143±2°C for ten minutes. Concentration was estimated at 575 mμ.

2) *Identification of extracted 2, 4, 5-T*

2, 4, 5-T in the urine was identified by the color reaction with chromotropic acid and by the spectrophotometric measurement of chloroform extracts. Gas-chromatographic identification was accomplished after methyl ester of 2, 4, 5-T with boron trifluoride being made[5]. Experimental conditions of gas-chromatographic analysis were shown in the foot note of Fig. 2.

3) Estimations of 2, 4, 5-T in the surroundings of a chemical manure factory

2, 4, 5-T in the surroundings was sampled with a high volume air sampler (Gelman, Type A). 2, 4, 5-T at the breathing point of the workers was sampled with a personal air sampler with a glass fiber filter. After sampling, filter papers were removed into a continuous extractor, containing approximately 100 ml of chloroform. 2, 4, 5-T, extracted in chloroform was estimated with the method mentioned above.

Results

1) Determination of 2, 4, 5-T in the urine

For a preliminary experiment, 500 and 100 μg of 2, 4, 5-T had been added to 100 ml of the urine of an adult and the urine was analyzed to obtain the recovery rates as high as 95.6, 92.1% respectively.

2) Identification of extracted 2, 4, 5-T

A peculiar wine-purple color developed when the extract was heated with chromotropic acid in the presence of concentrated sulfuric acid. The absorption spectrum of the color is presented in Fig. 1, showing the maximum absorption rate at 575 mμ. Spectrophotometric measurement of chloroform solution, one step before the last of the procedure,

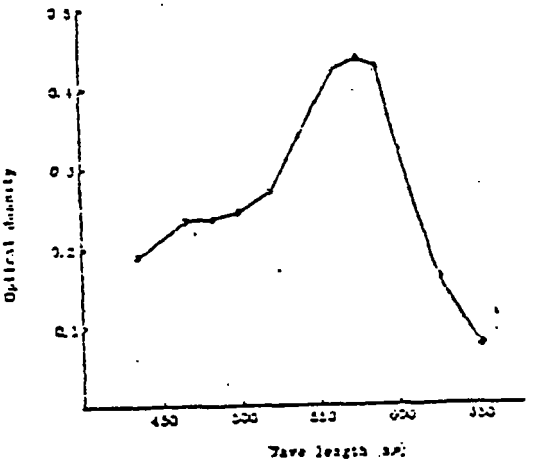


Fig. 1. Absorption spectrum of 2, 4, 5-T extracted from the urine when reacted with chromotropic acid at 143 to 145°C for 10 minutes.

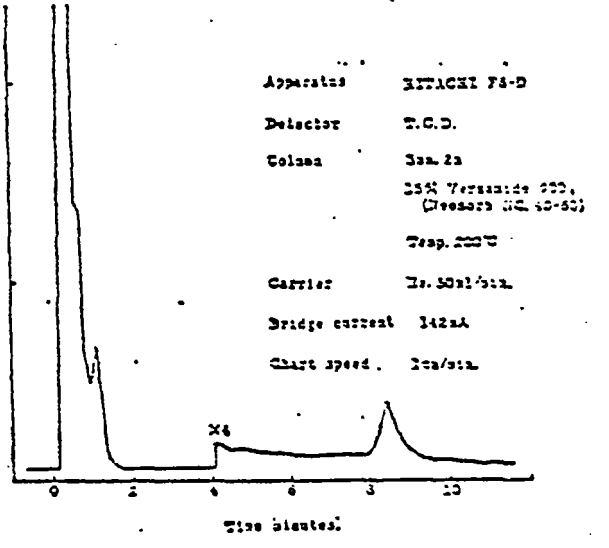


Fig. 2. Gas-chromatogram of 2, 4, 5-T obtained from the urine.

yielded the characteristic absorption curve of 2, 4, 5-T. The result of gas-chromatographic analysis is represented in Fig. 2, on which the sample coincided with pure chemicals in retention time of about 3 minutes. It is clear from these observations that 2, 4, 5-T was excreted in the urine in an intact form.

3) Distribution and elimination of 2, 4, 5-T

As will be seen in Fig. 3, the plasma concentra-

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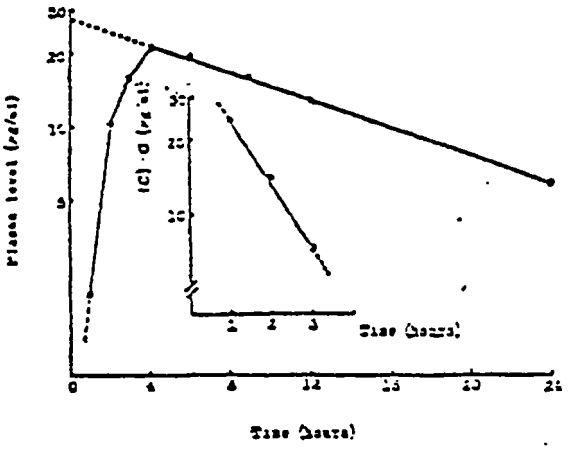


Fig. 3. Concentrations of 2, 4, 5-T in the blood plasma.

tion of 2, 4, 5-T rose rapidly during the first 3 hours and the peak plasma level (21.1 $\mu g/ml$) was attained at 4th hour after administration. The semi-logarithmic concentration-time curve was linear after 4 hours' post treatment, indicating a first order elimination rate with a gradient of -0.055 obtained with the method of least squares. Zero-time plasma level (28.0 $\mu g/ml$) was obtained by extrapolating the linear part of the plot.

The differences between the observed concentrations (C) and the respective ones obtained from extrapolation ((C)) were able to be plotted on a line with a gradient of -0.505, which indicate that the absorption is also of the first order.

The excretion of 2, 4, 5-T in the urine after a single oral administration was represented in Fig. 4 and Fig. 5. A fairly large amount of 2, 4, 5-T was recovered from the initial sample but only about 45% of original dose was found in the urine collected during the first 24 hours after administration. Thirty-six hours after treatment, 60% had been

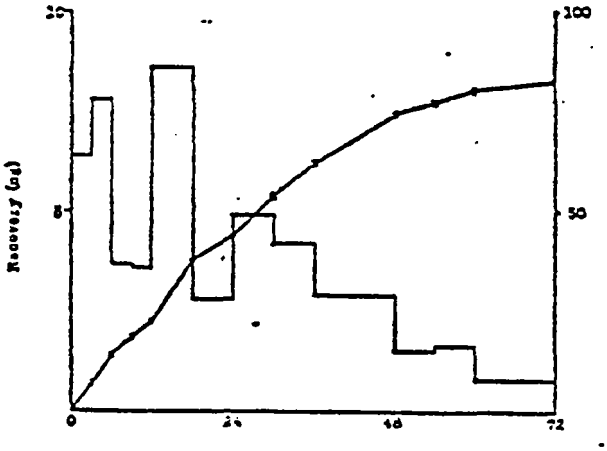


Fig. 4. Excretion of 2, 4, 5-T in the urine.

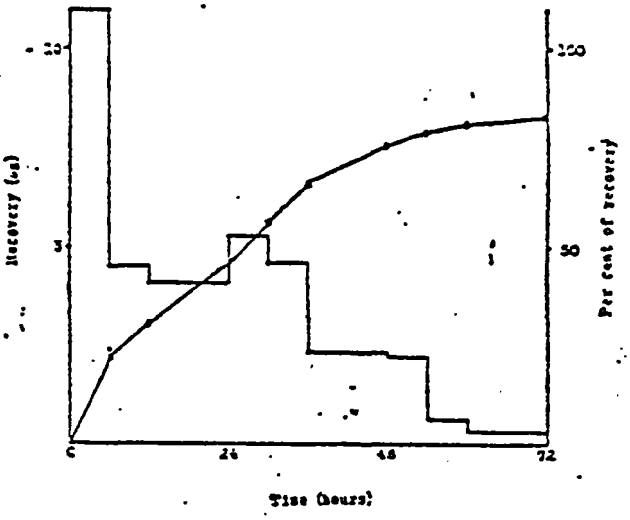


Fig. 5. Excretion of 2, 4, 5-T in the urine.

recovered and at the end of 72 hours more than 80% was recovered.

4) Estimations of 2, 4, 5-T in the urine as a practical trial

Table 1 demonstrates that a relatively small amount of 2, 4, 5-T was found in the urine of workers. In some of them, it was impossible to detect it with this method. The concentrations of 2, 4, 5-T in the working area were listed in Table 2 and Table 3. None but the value obtained in the weighing box of 2, 4, 5-T exceeded the recommended value by A.C.G.I.H., which is 10 mg/m^3.

Discussion

Two major bands of absorption by the phenoxyacetic acids are found in ultraviolet region[3]. The one band occurring in the region of 220-230 $m\mu$ is probably due to the carbonyl group of the side chain, while the other occurring around 280 $m\mu$ is due to the benzen ring. Nielsen[7] analyzed the concentration of 2, 4-D within the range of 200-350 $m\mu$. In the method, which relies upon the absorp-

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Table 1. 2, 4, 5-T in the urine of workers of a chemical manure factory.

Date	Name	Age	Sex	Urine volume (ml/day)	2, 4, 5-T (mg/day)
Apr. 9	A. M.	23	Male	2,050	1.5
—	S. S.	30	Male	1,400	—
	Y. I.	50	Male	1,570	—
	T. Xi.	40	Female	1,020	1.3
	S. H.	25	Female	1,020	1.2
	T. T.	20	Male	370	0.5
Apr. 15	A. M.	28	Male	2,000	3.5
	S. S.	30	Male	2,220	—
	Y. I.	60	Male	2,400	—
	S. H.	25	Female	1,250	—
	T. N.	33	Female	1,750	2.7
	S. K.	21	Male	1,400	3.6
Apr. 16	T. Xi.	40	Female	750	1.0*
	Y. I.	60	Male	1,670	—
	S. S.	30	Male	3,020	—
	S. N.	41	Male	1,470	2.2
	T. Ko.	58	Female	450	3.5*
Apr. 22	Y. O.	38	Female	250	—
	S. S.	30	Male	850	—
	T. Ki.	40	Female	150	1.9*
	Y. I.	60	Male	1,150	—

* milligrams per liter.

Table 2. Concentrations of 2, 4, 5-T in the surroundings of a chemical manure factory.

Sampling points	Concentrations*
Weighing box	15.4
Inlet of mixer	1.37
Outlet of mixer	0.82
Center of work-room	0.62

* milligrams per cubic meter.

Table 3. Concentrations of 2, 4, 5-T at the breathing location of workers.

Workmen	Concentrations*
A	0.21
B	0.31
C	0.67
D	0.38

* milligrams per cubic meter.

tion in the ultraviolet region, it is rather unsatisfactory to measure the concentrations lower than 1 mg/100 ml because of the low absorption rate. Using the color reaction with chromotropic acid, I can measure concentrations lower than that accurately.

On the other hand, industrially manufactured 2, 4, 5-T seems to be inevitably contaminated with 2, 4, 5-trichlorophenol, the precursor of 2, 4, 5-T, or other chlorophenol derivatives. As long as the measurement is carried out in the ultraviolet region, at 288 mμ, the contaminants possessing the benzen ring can not be excluded. Chromotropic acid enhances not only sensitivity but also specificity.

The development of the color depends upon a reaction between 2, 4, 5-T and chromotropic acid in the concentrated sulfuric acid. It is said that the heating of 2, 4, 5-T in the presence of concentrated sulfuric acid results in the formation of formaldehyde which then reacts with the chromotropic acid[10]. The reaction, highly sensitive to a small amount of phenoxyacetic acid, is relatively nonspecific. A number of other compounds such as acetic acid, fats and waxes may react and give a similar color. But in this study, samples free from 2, 4, 5-T did not develop the characteristic purple color. Sometimes when the samples were heated above 150°C, they developed amber color, which was bleached by a stannous chloride solution. Since the wine-purple color of chromotropic acid had the maximum absorption at 575 mμ, the concentration was estimated in this region.

In preliminary experiments, the recovery of 2, 4, 5-T, added to the urine of an adult, was satisfactory and that of 2, 4, 5-T, added to the homogenate of the organs of rats, was shown elsewhere[15].

The identity of 2, 4, 5-T, extracted from the urine, was judged by the following criteria: a characteristic color reaction with chromotropic acid representing the absorption maximum at 575 mμ, coincidence of the absorption maximum with pure 2, 4, 5-T in the ultraviolet range at 288 mμ, and coincidence of a retention time in gas-chromatographic analysis. The acetic acid of the side chain was demonstrated by the positive color reaction with chromotropic acid. The benzene ring was investigated spectrophotometrically in the ultraviolet region. Not only the intactness of ether bondage but also the intactness of the chlorine of the side chain were confirmed by gas-chromatograms. It can be concluded from these observations that ingested 2, 4, 5-T was excreted in the urine unchanged.

Orally administered 2, 4, 5-T was readily absorbed as indicated by the observation that the peak plasma

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concentration was attained within a few hours after a single administration. Linearity of the semi-logarithmic concentration-time curve in its terminal course indicates that 2, 4, 5-T was eliminated from the blood plasma at the first order elimination rate. On the other hand, non-linear part of the plot represents the absorption of 2, 4, 5-T from the digestive tract. The absorption rate could be estimated indirectly by plotting the differences between the observed concentrations and respective ones obtained from extrapolation on semi-logarithmic scale against time, which stands for the absorption rate[7].

Distribution and elimination of 2, 4, 5-T have rarely been investigated. Only Kurt-Ernst[3] reported that the plasma concentration time curves as well as the plasma half-life values of 2, 4, 5-T were similar to those obtained with 2, 4-D in experimental animals. Accordingly, it is of interest to compare the fate of 2, 4, 5-T in man with that of 2, 4-D in experimental animals. Distribution volume can be estimated from zero-time plasma level and the dosage given at 7.9% of the body weight, approximately as large as the total blood volume. It is said that 2, 4-D could regularly be detected in blood cells in concentrations ranging 10 to 20% of the corresponding plasmas. Based on these findings, two thirds of the orally administered 2, 4, 5-T would be distributed in the blood in earlier stages of elimination.

Clark[11] described in his paper that the radioactivity of labelled 2, 4-D rose rapidly in the blood plasma of sheep during the first half-hour, reached the peak after 1¼ hours and diminished rapidly thereafter. About 15% of the original dose was excreted in the urine in the first 1¾ hours. Eight and a half hours after treatment, 50% had been recovered in the urine and by the end of 28 hours, over 90% of the dose had been excreted. In contrast to these observations of Clark, the peak plasma level of 2, 4, 5-T was attained 4 hours after administration and the plasma level declined gradually, indicating the plasma half-life as 11 hours. Within 24 hours, only 45% of the original dose was found in the urine of volunteers. Low elimination rate, therefore, would make 2, 4, 5-T more toxic in man than in experimental animals.

It is mighty favorable for prevention and treatment of 2, 4, 5-T intoxication that the agent is excreted in the urine unchanged. The intake of 2, 4, 5-T could be estimated by urinalysis. As a practical trial, the urinalysis of the workers was performed to find out that a comparatively small amount of 2, 4, 5-T was recovered. In some of them, the agent could not be detected with the method mentioned above. It is to be accounted for by the low environmental 2, 4, 5-T concentrations, which were due to several kinds of improvement of operations. Furthermore, workers had been provided with such protectors as gloves, masks, hoods and protective suits and they took a bath at the end of duty. In the case of a well-controlled operation, the urinary excreations were occasionally lower than 1 mg/day. Thus, it would be inadequate to detect 2, 4, 5-T with the method mentioned, if the chronic toxicity of 2, 4, 5-T could not be ruled out in such levels.

Summary

To clarify the fate of 2, 4, 5-T in the human body, volunteers took 100 to 150 mg of 2, 4, 5-T orally. Blood and urine samples were collected and analyzed to find out that

1) orally administered 2, 4, 5-T was readily absorbed and eliminated gradually from the blood plasma, showing a first order elimination rate,
2) more than 80% of orally administered 2, 4, 5-T was excreted in the urine in an intact form within 72 hours, and
3) elimination from the blood plasma and excretion in the urine by man were slow, as compared with those in experimental animals.

According to the method elaborated, 2, 4, 5-T in the urine of workers of a chemical manure factory was analyzed.

Acknowledgement

The author wishes his appreciation to Dr. Ueda and Mr. Tarumi for the advice and encouragement they have made during the course of this study.

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除草剤 2,4,5-トリクロロフェノキシ酢酸 (2,4,5-T) のヒトの体内における運命を知る目的で，これを経口的に投与し，血中濃度の推移，尿中排泄量を検討した．

生体試料中の 2,4,5-T の抽出定量には Nielsen らの方法を改良し，強酸性下における 2,4,5-T とクロモトロープ酸との呈色反応を利用して定量した．

成人男子に 150 mg の 2,4,5-T を投与すると，すみやかに血液中の濃度は高まり，投与後4時間で最高値 21.1 μg/ml に達し，以後減衰に低下した．血液からの消失速度は一次であり，生体学的半減期は11.5時間と計算された．

尿中より抽出した物質を次の三つの方法で検討した結果，2,4,5-Tはヒトの体内において分解および抱合等の質的変化をうけずに尿中に排泄されてくることがわかった．

1) 強酸性下においてクロモトロープ酸と反応し特有なブドー酒色に呈色した．

2) 抽出物のクロロホルム溶液が紫外部において最高の吸収を示す点は 288 mμ にあり標準品のそれと一致した．

3) 抽出物を三弗化硼素でメチルエステルとしてからガスクロマトグラフィーで検討した結果，保持時間は約8分であり標準品のそれと一致した．

成人男子2名に 100 mg の 2,4,5-T を経口投与し，72時間にわたって採尿し尿中に排泄された2,4,5-Tを定量した．投与後24時間で投与量の約45%，48時間で約75%，72時間で80%以上が排泄された．これを実験動物における文献成績と比較すると，ヒトの場合には排泄により長時間を要するといえる．

これらの知見に基づいて産業現場で2,4,5-Tの体内侵入量を推定するための一段階として，ある化学肥料工場作業者の24時間尿について尿中2,4,5-Tを測定した．作業場環境中の 2,4,5-T の濃度は 0.62 mg/m³~15.4 mg/m³ であり，測定した延21名中最高値は 3.6mg/day であった．また約半数の尿中ではこの方法によって2,4,5-Tを検出することができなかった．

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used at 4 kg/ha preemergence, did not decrease the NO_3^-, NH_3, and P_2O_5 levels in the soil. It stimulated the activity of the soil microflora and increased the content of soil nutrients. A 30–50% increase in N content was noted in the presence of the herbicide. On Treflan-treated castor beans the yield of seeds was 88% higher than with the control. The content of N and P in plants cultivated in the presence of Treflan was higher than in the control. A. Radecki

120331s Use of herbicides on turnip plantings. Puzinaite, I. (USSR). *Khim. Sel. Khoz.* 1970, 8(2), 131–2 (Russ). Dacthal 4–8, diphenamid 3–6, trifluralin 2–4, and amiben 2–5 kg/ha were tested in 3-year field expts. against weeds in turnips planted in sod-podzols (pH 5.3–6.5). The effect of these herbicides on turnip yield were compared with a double hand weeding as the control. The yield was always less than in the control. The max. yield decrease (30 and 29%, resp.) was with 4 kg dacthal and 5 kg amiben/ha. With 3 kg diphenamid and 4 kg trifluralin/ha a min. yield decrease (10 and 11%, resp.) was obtained. Diphenamid was also most effective, even at 3 kg/ha, against *Raphanus raphanistrum* or *Stellaria media*, killing 73.3% when before thinning and 32% before harvest. Dacthal and trifluralin were less effective, and low levels of amiben had no effect; at 5 kg/ha amiben was toxic to turnips. J. Malek

120332t Preharvesting chemical drying of fodder lupine in the western Ukraine. Chernobai, N. Kh.; Proskura, I. P.; Mel'nichuk, P. D.; Bugaiskaya, Z. Ya. (USSR). *Khim. Sel. Khoz.* 1970, 8(2), 133–6 (Russ). Preharvest defoliants and desiccants for fodder lupine were tested in 3-year field expts. The most effective chems. (dose in kg/ha given) were: NH_4SCN (2, 5, 10, 25), NaSCN (2, 5, 10, 25), dinitro-*o*-cresol (1, 2, 5, 10), NaOH (5, 10, 25, 50), Reglone (0.5, 1.0, 2.5, 5.0), and Gramoxone (0.5, 1.0, 2.5, 5.0). Spraying of NaSCN or NH_4SCN 10–20, dinitro-*o*-cresol 2.5–5.0, and Gramoxone or Reglone 1.5–2.5 kg/ha accelerated seed maturation by 3–17 days, increased seed germinating by 9–13%, and increased seed crude protein by 0.4–1.6%. J. Malek

120333u Results of testing biennial weed control in wheat with new herbicides based on s-triazines, substituted ureas, and fluorenol. Chiapparini, L.; Soldini, G. B. (Sez. Patol., Osserv. Malattie Piante, Milan, Italy). *Notiz. Mal. Piante* 1969, No. 80–81, 209–48 (Ital). Excellent weed control with min. phytotoxicity were obtained with terbutrin [2-(*tert*-butylamino)-4-(ethylamino)-6-methylthio-*s*-triazine] applied preemergence at 1.5 kg of active ingredient/ha, metoxuron [*N*-(3-chloro-4-methoxyphenyl)-*N'*,*N'*-dimethylurea], neburon [*N*-butyl-*N'*-(3,4-dichlorophenyl)-*N*-methylurea], and prepn. 5633 (*N*-benzothiazolyl-*N*,*N'*-dimethylurea) postemergence at, resp., 3.2 or 4.8, 3.0 or 3.6, and 1.4 kg active ingredient/ha. Felix Saunders

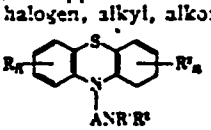
120334v Toxic effect of some herbicides on fish and aquatic invertebrates. Belyavskaya, L. I.; Konstantinov, A. S.; Konstantinova, N. S. (USSR). *Vidovoi Sostav, Ekol. Prod. Gidrobiontov Volgograd. Vodokhran.* 1969, 93–8 (Russ). Edited by Konstantinov, A. S. Izd. Saratov. Univ.: Saratov, USSR. For the prepn. of huge water-reservoir beds, woods and shrubs are often destroyed with herbicides e.g., the Bu ester of 2,4-D, the Bu ester of 2,4,5-T, Foredex 75, and Fortex (aq. emulsions of 2,4-D and 2,4,5-T, resp.). After 20–30 min of contact of these emulsions at 60–120 mg/l., crayfish (*Simacephalus vetulus*) died; on contact with aq. emulsions contg. 10 mg/l., they survived for 5 days. Of all herbicides tested on invertebrates, which serve as food for fish, 2,4,5-T was the most toxic, and it killed them all at 2 mg/l. These herbicides at 4 and 6 mg/l. were toxic to 7 types of fish when added to Volga water at 14–18° for 1 day. Solar oil was also toxic to the fish and the fish feed. At their present level of use 6 kg/ha of shrubland, the concn. of herbicides in water in the studied reservoirs will soon reach fatal levels to the fish and their food. However, if the herbicide treatment were limited to 0.01–0.1% of the total bed-area of the reservoirs, the resulting concns. in the water will be harmless to the fish and their bio-food. S. K. Raman

120335w Combined effect of trifluralin and MSMA [monosodium methanearsonate] on Johnsongrass control in cotton. Kleifeld, Y. (Volcani Inst. Agr. Res., Bet Dagan, Israel). *Weed Sci.* 1970, 18(1), 16–18 (Eng). Preplanting application of trifluralin reduced the no. of mono-Na methanearsonate (MSMA) applications needed for the control of Johnsongrass (*Sorghum halepense*). The combined effect enabled weed control to be completed before cotton (*Gossypium hirsutum*) began to bloom, and hence without causing damage to the crop. RCKZ

120336x Metabolism of chlorbromuron-^{14}C in corn and cucumber. Nashed, R. B.; Katz, S. E.; Ilnicki, Richard D. (Coll of Agr. and Environ. Sci., Rutgers Univ., New Brunswick, N.J.). *Weed Sci.* 1970, 18(1), 122–5 (Eng). The metabolic fate of root-applied, ^{14}C-carbonyl-labeled, 3-(3-chloro-4-bromophenyl)-1-methoxy-1-methylurea (chlorbromuron) was investigated in tolerant corn (*Zea, mays* var N.J. 9) and susceptible cucumber (*Cucurbita sativus*, var Marketer) in a time-course study. The major metabolite found in corn shoots and roots was the nonphytotoxic 3-(3-chloro-4-bromophenyl)-1-methoxy-urea. Evidence of binding was found in both shoots and roots of corn. No evidence was found for the metabolism of chlorbromuron in cucumber; however, some binding occurred in both shoots and roots. RCKZ

120337y Effect of the ratio of soil to water on the adsorption of linuron and atrazine. Grover, Rajbans; Hance, R. J. (Can. Agr. Res. Sta., Regina, Sask.). *Soil Sci.* 1970, 109(2), 136–8 (Eng). Begbroke soil was prepd. by air-drying and passing through a 10 mesh sieve. The soil contained 1.93% C, 15.6% clay, 18.4% silt, and 66.0% sand. Its water holding capacity was about 24%. The pH of a 1:1 (soil:water) suspension was 7.1. Five herbicide concns. were used in the range of 5–25 ppm linuron and 0.5–5 ppm atrazine for the 1:10 (soil:water) ratio; 5–25 ppm linuron for the 1:1 ratio; and 15–45 ppm linuron and 5–20 ppm atrazine for the 4:1 ratio. The linuron solns. were made in 0.1 *M* $CaCl_2$ and the atrazine solns. contained no $CaCl_2$. Adsorption isotherms were linear in each case and comparison was made using the Freundlich relation with values of *k* reported. There was a 5-fold increase in the adsorption of linuron at the 1:10 ratio (*k* 12.3) compared to the 4:1 ratio (*k* 2.7); adsorption in the 1:1 mixt. showed *k* 4.4. For atrazine the difference between slurry conditions (1:10) and the 4:1 ratio was approx. 3-fold, the *k* values being 0.8 and 2.1, resp. It was concluded that adsorption of herbicides under field conditions, where the soil aggregates are relatively large, may not be as high as those estd. under slurry conditions. There may be variations in the extent of adsorption of a herbicide within 1 field if aggregate size distribution varies. Peter Coad

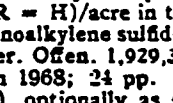
120338z Phenothiazines as plant growth regulants. Farbenfabriken Bayer A.-G. Fr. Demande 2,001,581 (Cl. A 01*n*, C 07*d*), 26 Sep 1969, Ger. Appl. 08 Feb 1968; 7 pp. Phenothiazines (I) (R, R^1 = halogen, alkyl, alkoxy, CF_3, alkylcarbonyl,



(I)

A represents alkylene, R^1, R^2 = alkyl or form together with the N atom a 5- or 6-membered ring; *n* = 0, 1, or 2) and their salts are used as plant growth regulants. Compns. with various concns. of I can act as growth stimulants, growth inhibitors, or herbicides. The compns. generally contain 0.1–95% I and are applied at the rate of 0.001–100 kg/ha. I may be applied to dicotyledons (cotton, beets, carrots, beans, potatoes, coffee, mustard, water cress, bedstraw, camomile, and galingosa) and monocotyledons (maize, rice, oats, barley, wheat, millet, sugar cane, cat's tail, spear grass, ray grass, and panic grass). G. P. Natus

120339a Imidazoles as herbicides. Draber, Wilfried; Falbe, Jurgen F.; Buechel, Karl H.; Korte, Friedrich W. A. G. K. (Shell Oil Co.) U.S. 3,501,286 (Cl. 71–92; A 01*n*), 17 Mar 1970, Appl. 29 Jun 1964; 4 pp. Imidazoles (I) where R is H, C_{1-18} alkyl, cyanoethyl, or allyl, and Y is H, C_{1-4} alkyl, CN, halogen,



(I)

or NO_2 were active as general preemergent herbicides at 10 lb/acre, while they selectively controlled broadleaf weeds at 2 lb/acre. Wettable powder, dust, and emulsifiable conc. compns. are given. A dust contained 4,5-dibromoimidazole 4, Mg stearate 1, kaolin 58, and gypsum 37 parts by wt. In field tests I(Y = Br, R = H) gave 99% control of broadleaf weeds at 2 lb/acre, but only temporary burning of grasses. Germination of weed seeds was prevented by incorporating 2–8 lb I (Y = Br, R = H) or I (Y = I, R = H)/acre in the soil. L. Tetzloff

120340u Dialkylaminoalkylene sulfide herbicides. Bordenca, Carl (SCM Corp.) Ger. Offen. 1,929,390 (Cl. A 01*n*), 19 Feb 1970, US Appl. 27 Jun 1968; 24 pp. β-Dialkylaminoalkylene sulfides $RSXNR^1R^2$ (I), optionally as stable salts thereof, are used; R = linear or branched C_{4-18} alkyl, preferably octyl and decyl branched or not, X = lower alkylene, preferably ethylene, R^1 and R^2 = same or different lower alkyl, preferable Et. β-Diethylaminoethyl *n*-octyl sulfide, β-diethylaminoethyl 3,7-dimethyloctyl sulfide, and β-diethylaminoethyl 4,6,8-trimethylnonyl sulfide, optionally as hydrohalides, are claimed. I were tested as pre- and post-emergence herbicides. Manfred Knaack

120341v Herbicidal Cymetrin [2-methylthio-4,6-bis(ethylamino)-s-triazine] and benzyl alkylthiocarbamate mixtures. Kimura, Ichiro; Sugiyama, Hironari; Kado, Masaru (Kumiai Chemical Industry Co., Ltd.) Ger. Offen. 1,943,983 (Cl. A 01*n*), 12 Mar 1970, Japan. Appl. 28 Aug 1968; 07 Jan 1969; 24 pp. A mixt. of Cymetrin (I) and thiocarbamates (II) II (R = R^1 = Et, X = 4-Cl) ($b_{0.15}$ 127–31°) 7%, I 1.5%, Na ligninsulfonate 2%, wood ashes 5%, and bentonite 84.5% by wt. were ground and mixed to homogeneity, kneaded with water, and granulated to granules of 14–32 mesh. The herbicidal mixt.

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DOW CHEMICAL U.S.A.

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J. H. Karwal

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Simultaneous gas chromatographic determination of 2,4-D and dicamba in human blood and urine

The compounds 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methoxy-3,6-dichlorobenzoic acid (dicamba) are herbicides commonly used to control a variety of broadleaf plants. An acute poisoning case was recently investigated by the Hawaii Community Study on Pesticides in which the victim intentionally ingested a formulation containing 2,4-D and dicamba. The occurrence of this attempted suicide created a need for a rapid and sensitive method for detecting these compounds in small amounts of human blood and urine.

Several methods for detecting 2,4-D residues in a variety of substrates were considered for the analysis of 2,4-D and dicamba in human blood and urine[1-3]. While generally sensitive, these methods were neither rapid nor applicable to the relatively small samples available.

The Hawaii Community Study on Pesticides uses the method of BEVENUE *et al.*[4] to determine pentachlorophenol (PCP) residues in human blood. In an evaluation done by this laboratory[5], the procedure was shown to be rapid, accurate, sensitive and reproducible. Because 2,4-D and dicamba display chemical characteristics similar to those of PCP, these compounds are also detected by the procedure.

This note describes the application of the method of BEVENUE *et al.*[4] to the simultaneous determination of 2,4-D and dicamba in human blood and urine.

Experimental

Solvents and reagents. The following solvents and reagents were used: benzene, hexane, isooctane (Mallinckrodt Nanograde Reagents), used as received; 0.1 *N* H_2SO_4, benzene extracted; 2,4-dichlorophenoxyacetic acid; 2-methoxy-3,6-dichlorobenzoic acid; N-methyl-N'-nitro-N-nitrosoguanidine, supplied by Aldrich Chemical Co., Milwaukee, Wisc.

The diazomethane solution[6] was prepared as follows: hexane (25 ml) and 2 ml of 20% aqueous NaOH were combined in a 50-ml erlenmeyer flask and 0.5 g of N-methyl-N'-nitro-N-nitrosoguanidine was added in small increments. When the reaction was complete, the hexane layer was decanted into a small glass bottle equipped with a Teflon-lined screw cap. The diazomethane solution was kept in a freezer for up to one week. Because diazomethane is a skin irritant and a carcinogen, its preparation and use was confined to a well-ventilated hood.

Standard solutions. Stock solutions of 2,4-D and dicamba in benzene were prepared, each containing 1 mg of compound per ml of solvent. In separate 100-ml volumetric flasks 1.0 ml of each stock solution was treated with 0.5 ml of diazomethane solution and allowed to stand for 15 min. The solutions were then diluted to 100 ml with isooctane, thus giving solutions which contained 10 μg/ml of each compound as its methyl ester. From these solutions, working standards were prepared in appropriate concentrations.

Apparatus. Gas chromatographic determinations were made on a MicroTek MT 220 gas chromatograph equipped with a ^{3}H electron capture detector and a 0.32 cm I.D. × 180 cm borosilicate glass column packed with a mixture of 4%

5667

SE-30 and 6% QF-1 on Chromosorb W (AW-DMCS), high-performance grade, 80–100 mesh.

The instrument conditions were: inlet temperature 220°, detector temperature 208°, column temperature 190°; nitrogen flow rate 85 ml/min.

The 25-ml evaporative concentrator tube was Kontes No. 570050-2526, the evaporative concentrator column Kontes No. 569251-0324.

Extraction. One milliliter of blood serum or 5 ml of urine were combined with 20 ml of 0.1 *N* H_2SO_4 and 12 ml of benzene in a 125-ml glass-stoppered erlenmeyer flask equipped with a 1-in. Teflon-coated magnetic stirring bar and mixed for 20 min at 50° on a heated stirplate. After cooling, the contents of the flask were transferred to a 35-ml centrifuge tube and centrifuged for 15 min to effect phase separation. The benzene (top) layer was transferred to a 25-ml evaporative concentrator tube, and the aqueous phase was re-extracted three times with 5-ml portions of benzene. With each wash the mixture was shaken vigorously for 1 min, and the phases were separated by centrifugation. The benzene extracts were combined in the 25-ml evaporative concentrator tube, a boiling chip was added, an evaporative concentrator column was attached, and the combined extract was concentrated to 2 ml in a water-bath at 100°.

Methylation. Diazomethane solution (0.2 ml) was added to the concentrated extract, and the mixture was allowed to stand for 15 min. Excess diazomethane was then removed by gently bubbling dry nitrogen through the extract until the yellow color disappeared, and the volume was adjusted to 10.0 ml with isooctane.

Gas chromatography. The methylated extract was diluted as necessary, and 2 to 8 μl were injected into the gas chromatograph. Following each extract injection, an amount of standard which gave a response of similar peak height (± 10%) was injected.

Results and discussion

The 2,4-D and dicamba levels in the poisoning case samples are given in Table I. The results of a recovery study to determine the applicability of the procedure to the analysis of human blood and urine for 2,4-D and dicamba residues are given in Table II. The samples were fortified, prior to extraction, with 2,4-D and dicamba acids at levels which compared generally with the wide range of concentrations found in the poisoning case samples.

In all cases responses to 2,4-D and dicamba (as their methyl esters) were quantitated by comparison with standard responses in terms of peak height. By comparing responses with peak heights differing by 10% or less, quantitation problems associated with lack of linearity and changing sensitivity, sometimes encountered with electron capture detectors, were minimized.

The limits of detectability in 1 ml of blood serum were 0.03 p.p.m. for dicamba and 0.05 p.p.m. for 2,4-D. In 5 ml of urine the limits of detectability were 0.01 and 0.02 p.p.m. for dicamba and 2,4-D, respectively.

Under the conditions given, dicamba and 2,4-D methyl esters had retention times of 1.2 and 1.0 min, respectively; PCP methyl ether had a retention time of 2.2 min. Because the three compounds are well separated on the gas chromatographic column, the procedure is useful for detecting any or all of the compounds in the same sample.

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5668

TABLE I

2,4-D AND DICAMBA LEVELS IN POISONING CASE SAMPLES

Sampling	Blood serum		Urine					2,4-D (p. p. m.)		Dicamba (p. p. m.)	
	Date[a]	Time	Date[a]	Time	to Date[a]	Time	Vol. (ml)	Serum	Urine	Serum	Urine
1	10/14	7:00 a.m.	10/14	11:00 a.m.	10/14	3:00 p.m.	740	1006	115.2	46.4	215.0
2	10/14	3:00 p.m.	10/14	3:00 p.m.	10/14	11:00 p.m.	375	1031	271.0	22.3	280.3
3	No sample taken		10/14	11:00 p.m.	10/15	9:00 a.m.	1230	-	151.5	--	17.5
4	10/15	9:00 a.m.	10/15	9:00 a.m.	10/15	4:00 p.m.	--	911.9	762.0	8.52	12.8
5	10/15	4:00 p.m.	10/15	4:00 p.m.	10/15	11:00 p.m.	775	931.2	590.8	8.12	1.05
6	10/15	11:15 p.m.	10/15	11:00 p.m.	10/16	7:45 a.m.	1170	736.4	781.3	6.09	1.22
7	10/16	7:50 a.m.	10/16	7:45 a.m.	10/16	4:15 p.m.	720	618.6	408.9	4.17	1.08
8	10/16	4:15 p.m.	10/16	4:15 p.m.	10/16	11:00 p.m.	815	461.0	1333	3.06	0.95
9	10/16	11:00 p.m.	10/16	11:00 p.m.	10/17	9:00 a.m.	740	383.1	1000	2.51	1.35
10	10/17	9:00 a.m.	10/17	9:00 a.m.	10/17	5:00 p.m.	310	361.3	1412	1.07	1.06
11	10/17	5:00 p.m.	10/17	5:00 p.m.	10/18	10:00 a.m.	1640	72.4	400.6	1.30	0.80
12	10/18	10:00 a.m.	10/18	10:00 a.m.	10/18	4:30 p.m.	910	5.23	77.9	0.17	0.51
13	10/18	4:30 p.m.	10/18	4:30 p.m.	10/19	9:45 a.m.	1400	1.69	8.60	0.23	0.10
14	10/19	9:45 a.m.	10/19	9:45 a.m.	10/19	3:00 p.m.	450	1.78	3.88	0.15	0.25
15	No sample taken		10/19	3:00 p.m.	10/20	9:00 a.m.	2150	--	4.51	-	0.12
16	10/20	9:00 a.m.	10/20	9:00 a.m.	10/21	8:30 a.m.	2420	1.26	2.90	0.15	0.06
17	10/21	8:30 a.m.	10/21	8:30 a.m.	10/22	9:30 a.m.	3710	0.38	1.58	0.15	<0.01
18	10/22	9:30 a.m.	10/22	9:30 a.m.	10/23	8:00 a.m.	2000	0.16	1.71	0.03	<0.01
19	10/23	7:45 a.m.	10/23	8:00 a.m.	10/24	8:45 a.m.	1405	0.30	0.51	0.07	<0.01
20	10/24	8:15 a.m.	10/24	8:45 a.m.	10/25	1:00 p.m.	2060	0.88	0.71	0.06	0.03
21	10/25	1:00 p.m.	10/25	1:00 p.m.	10/27	10:45 a.m.	3800	0.38	0.25	<0.03	0.01
22	10/27	10:45 a.m.	10/27	10:45 a.m.	10/29	8:00 a.m.	2110	0.28	0.12	<0.03	<0.01
23	No sample taken		10/29	8:00 a.m.	10/31	1:00 p.m.	3030	--	1.15	--	<0.01
24	10/31	1:00 p.m.	10/31	1:30 p.m.	11/3	7:30 a.m.	1360	0.31	1.13	<0.03	<0.01
25	11/7	9:00 a.m.	11/7	6:00 a.m.	11/8	6:00 a.m.	1300	0.56	0.81	<0.03	<0.01

[a] 1968

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5669

TABLE II

RECOVERY OF 2,4-D AND DICAMBA ADDED TO HUMAN BLOOD SERUM AND URINE

Sample[a]	*Fortification level (p. p. m.)*		*Found (p. p. m.)*		*% recovery*	
	Dicamba	*2,4-D*	*Dicamba*[b]	*2,4-D*[b]	*Dicamba*	*2,4-D*
Urine	—	—	< 0.01[c]	< 0.02[c]	—	—
	65.0	685	68.8	779	100	114
	13.0	137	12.5	132	96	96
	1.30	13.7	1.15	11.6	88	85
	0.13	1.37	0.11	1.35	85	98
	0.013	0.137	0.01	0.15	77	109
	0.006	0.068	< 0.01[c]	0.07	—	103
Blood serum	—	—	< 0.03[c]	< 0.05[c]	—	—
	65.0	685	61.0	636	94	93
	6.50	68.5	5.91	68.9	91	101
	0.65	6.85	0.55	5.90	85	87
	0.065	0.685	0.07	0.60	108	88
	0.006	0.068	< 0.03[c]	0.06	—	88

[a] Each sample a single analysis.
[b] Detected as their methyl esters.
[c] Limit of detectability.

No interfering responses were encountered in any of the samples analyzed; however, the use of the diazomethane solution in amounts greater than those indicated in the procedure can lead to interferences due to impurities in the reagent.

Using this procedure, an experienced analyst can process approximately sixteen samples per day.

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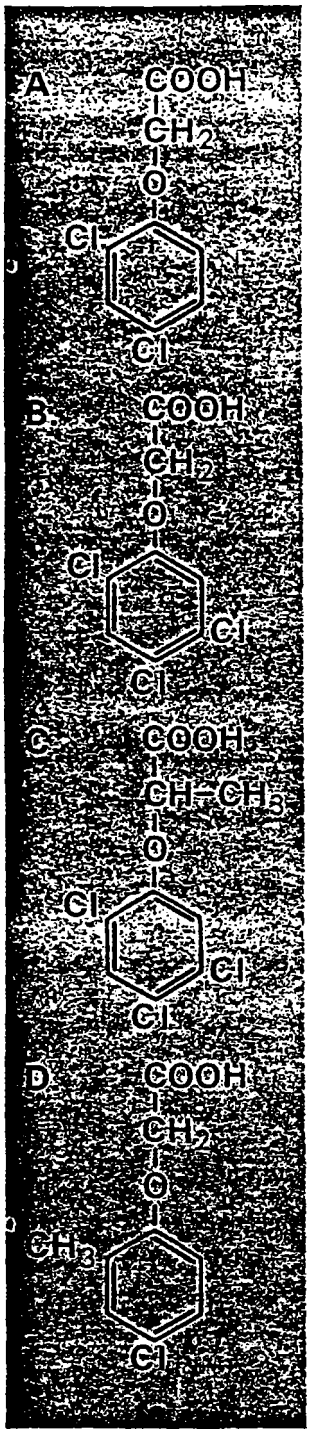
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A. 2,4-dichlorophenoxyacetic acid
B. 2,4,5-trichlorophenoxyacetic acid
C. 2-(2,4,5-trichlorophenoxy)-propionic acid
D. 2-methyl-4-chlorophenoxyacetic acid

The status of 2,4-D, 2,4,5-T, Silvex and MCPA Herbicides

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BACKGROUND

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was developed in the mid-forties and was the forerunner of a group of phenoxy herbicides that have been instrumental in the control of broadleaved weeds in food crops and undesirable brush species on industrial rights-of-way. In addition these products have contributed immeasurably to beef production by controlling weed and/or brush on pasture and rangeland, resulting in increased grass production and corresponding increase in carrying capacity for livestock.

Besides 2,4-D, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-(2,4,5-trichlorophenoxy) propionic acid (silvex or 2,4,5-TP), and 2-methyl-4-chlorophenoxy acetic acid (MCPA) are major phenoxy products of similar chemical structure, as shown below, but with unique characteristics of their own with respect to species controlled and crop selectivity.

CHRONOLOGY OF EVENTS

Over the years there has been considerable improvement in phenoxy herbicides and their use. Development of new formulations, performance information, crop safety, timing of application, spray equipment, toxicology, use hazards, and environmental implications have contributed to both better product and specific directions for use.

These herbicides are not protected by patents, so seven commercial companies were manufacturing one or more of the four phenoxies (2,4-D, 2,4,5-T, silvex, MCPA) at the time when the USDA announcement to abolish the no residue status was issued on April 13, 1966. Basic manufacturers included: Diamond, Dow, Hercules, Monsanto, Rhodia (then Chipman), Thompson, and Thompson-Hayward. These companies joined to form the Industry Task Force on Phenoxy Herbicide Tolerances (ITFPHT). The chronology of major events associated with this has been as follows:

April 13, 1966

USDA announcement to abolish no residue status—industry must comply by obtaining tolerances for residues in all treated food and feed products and byproducts by December 31, 1970.

August 23, 1966

Industry Task Force on Phenoxy Herbicide Tolerances was formed to handle 2,4-D, 2,4,5-T, MCPA and silvex.

December, 1967

Submitted petitions to FDA for tolerance of 2,4-D, 2,4,5-T, silvex and MCPA covering all food crop uses listed at that time in the USDA Summary of Registered Agricultural Chemical Uses. Extension of registration was requested for uses of these herbicides in pasture and rangeland.

April, 1968

Industry Task Force advised of inadequacies, in the petitions plus requirement for information on all metabolites of the herbicides that might occur as residues in food.

September, 1968

Review of literature on metabolism submitted to resolve metabolite question.

During 1968 programs were established to determine on which crops additional work would be undertaken and what specific projects would be done by each company in the Task Force.

October 29, 1968

Industry Task Force requested extension for continued use of 2,4-D, 2,4,5-T, silvex and MCPA on pastures and rangeland. Extension was granted until January 1, 1971.

January 31, 1969

Use of 2,4-D, 2,4,5-T and silvex on aquatic sites extended to January 1, 1970.

Crop samples for residue analyses were collected during summer and fall of 1969.

Protocols for milk and meat studies were established during 1969. It was decided that analyses of animal tissues would include the phenol corresponding to each phenoxy compound. Dow handled dairy cattle feeding and milk analysis for all four phenoxies and began feeding February, 1970.

USDA was scheduled to handle feeding of phenoxies to beef cattle and sheep during winter, 1969-1970.

5672

October 29, 1969
The Office of Science and Technology issued statement on teratogenic hazard of 2,4,5-T based on work by Bionetics Research Laboratory.

December 22, 1969
Phenoxy registration for rangeland use extended until January 1, 1971, except for 2,4,5-T. Dow undertook feeding of 2,4,5-T to beef animals.

December 31, 1969
Petition for tolerances of 2,4,5-T in food crops was withdrawn by Industry Task Force since tolerances could not be established by January 1, 1970, the deadline set by the Office of Science and Technology.

January 19, 1970
Registration of 2,4-D, silvex and MCPA extended by USDA for use on food crops until January 1, 1971.

March 4, 1970
Registration of 2,4,5-T was also extended until January 1, 1971 for use on apples, blueberries, grains, pastures, rangeland, rice and sugarcane.

April 15, 1970
Results of additional work on teratogenic properties of 2,4,5-T prompted suspension by USDA of 2,4,5-T for aquatic and home uses.

May 1, 1970
Cancellation by USDA of 2,4,5-T for use on food crops. Uses on pasture, forests and industrial areas not affected.

May 28, 1970
Dow appealed the cancellation of 2,4,5-T for use on rice Hercules and Amchem also appealed cancellation for rice usage. Each company could appeal only crops listed on their labels for 2,4,5-T products. The appeal is to be reviewed by an Advisory Committee appointed by the National Academy of Sciences.

June 16, 1970
USDA began the beef feeding studies scheduled to have been done the previous winter.

November 24, 1970
Crop residue work completed. Milk analysis completed. Meat analyses underway. Completed data will be submitted as amendment to petitions prior to December 31, 1970.

To date, no official notice has been received concerning appointment of National Academy of Science Advisory Committee to review the appeal on the cancellation of rice.*

REGISTRATION STATUS

Tolerances have been established for 2,4-D in applies, barley, grapefruit, lemons, oats, oranges, pears, rye and wheat. There is also a tolerance for the sodium salt of 2,4-D in asparagus.

Data originally submitted with the petitions in December, 1967 is expected to be sufficient for 2,4-D in blueberries, cranberries, grapes and raspberries; for silvex in apples, pears and prunes; and for MCPA in peas. Data on residues in grass includes that from previous work and from 1970 residue samples additionally analyzed by Dow for support with respect to pasture and rangeland usage.

The Industry Task Force supported work for determining residues as shown in Table 1.

For virtually all of this work, new analytical methods had to be developed that would permit analyses down to 0.1 ppm phenoxy acid in crops and to 0.05 ppm acid or corresponding phenol in the animal tissues and milk.

For present registered uses residue analyses indicate < 0.2 ppm for all phenoxy acids in all crops at time of harvest.

The phenoxies were fed at levels of 30, 100 and 300 ppm for two weeks and 1000 ppm for three weeks in the total diet of dairy cows. Milk was collected and analyzed for residues. Grazing restrictions compatible with levels of phenoxies in milk as related to levels in forage remain to be determined. There was no evidence of accumulation of phenoxies in the cream.

At time of this writing, December 1, 1970, analyses are being run on samples of muscle, kidney, liver and fat of beef animals. Data are expected to be available prior to December 31, 1970.

Amendments to the petitions for tolerances for 2,4-D, silvex and MCPA will be submitted to the appropriate agency in charge, including uses in pasture and rangeland. The 2,4,5-T petition will be reactivated —deadline for this compliance will be accomplished prior to December 31, 1970.

TOXICOLOGY

Negligible residue tolerances can be obtained based on information from 90 day toxicology studies in two species of mammals. However, tolerances at higher (permissible) residue levels require two-year feeding studies on rats and dogs, plus fertility and reproduction studies on rats. At the time the phenoxy herbicides were

5674

* See update at end of text (1).

TABLE 1. Crops and tissues sampled for residue analysis (+) under the ITFPHT program.

	2,4-D	2,4,5-T	Silvex	MCPA
Corn				
Rice				
Flax				
Small Grains (Barley, Oats, Rye, Wheat)		Tolerances Granted		
Sorghum				
Sugarcane				
Milk				
Meat				

developed these long term feeding studies were not necessary, since these compounds were registered on a "no-residue" basis.

FDA has conducted two-year feeding studies on 2,4-D including reproduction and fertility. Dow has conducted two-year feeding studies on silvex but not reproduction or fertility studies.

Ninety-day feeding studies have been run on rats and dogs for 2,4-D, 2,4,5-T, silvex and MCPA. The no-ill effect levels are shown in Table 2.

Based on single oral doses in rats, 2,4-D, 2,4,5-T, silvex and MCPA are classed as "slightly toxic" with LD_{50} values ranging from three hundred to seven hundred mg/kg body weight.

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

The word teratology has recently become much more familiar. It was tied to 2,4,5-T when studies by Bionetics Research Laboratory implied that 2,4,5-T was teratogenic (producing malformed fetuses) in mice and rats. Subsequent studies have shown that a potential toxic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin, is responsible for the findings attributed to 2,4,5-T. The sample of 2,4,5-T employed in the Bionetics study contained 27 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Additional studies have shown that oral administration of 2,4,5-T containing < 1 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin produced no teratogenic effects on rats, rabbits or mice. The obvious concern is to produce 2,4,5-T without the contaminant.

The 2,3,7,8-tetrachlorodibenzo-p-dioxin can be formed in the manufacture of the precursor 2,4,5-trichlorophenol. The conditions required for its formation are high temperatures and basic conditions. This can occur in the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene to the trichlorophenol.

No detectable dioxins have been observed in 2,4-D. This is due to the fact that the precursor 2,4-dichlorophenol is made by direct chlorination of the phenol and not by alkaline hydrolysis of 1,2,4-trichlorobenzene.

To date analytical methods have been developed and validated for a method sensitivity of 0.5 ppm for 2,3,7,8-tetrachlorodibenzo-p-dioxin in 2,4,5-T acid. With proper manufacturing controls there is no problem in producing 2,4,5-T with no 2,3,7,8-tetrachloro-p-dioxin as indicated by these analytical methods.

THE FUTURE

By December 31, 1970 the Industry Task Force on Phenoxy Herbicide Tolerances will have furnished to FDA the supplemental residue data necessary for the continued evaluation of the petitions to establish negligible residue tolerances for 2,4-D, 2,4,5-T, silvex and MCPA on the appropriate food crops and meat and milk tissues. Registrations (USDA) are expected to remain in force.

TABLE 2: Results from 90-day subacute feeding studies of various phenoxy herbicides in rats and dogs.

	Approximate No-Ill Effect Levels (Mg/Kg/Day)			
	2,4-D	2,4,5-T	Silvex	MCPA
Rats	30	30	5-10	16
Dogs	10	5	7	8-10

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Data provided to date indicate no hazard and no significant residues in food crops. Toxicology data support the use claims. The proper manufacturing of 2,4,5-T will alleviate the problems associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin.

The USDA has recently stated that prohibiting the use of phenoxy herbicides would cost the U. S. farmers an additional $290 million to maintain current agricultural production. In addition, farmers and their families would have to work 20 million more hours to control the weeds without these herbicides. For this extra labor, the farmers would obtain no additional income".

Several hundred thousands of dollars have been expended over the past several years to prove the safety of phenoxy herbicides to man and his environment. From a scientific base the phenoxy herbicides can contribute economically, efficiently, and safely in the future for the control of broad-leaved weeds and brush on food crops, pasture, rangeland, and non-cropland areas as they have for over 20 years.

SIGNIFICANT CHRONOLOGICAL EVENTS SINCE PREPARATION OF MANUSCRIPT

As of January 23, 1971 several significant events have occurred.

(1) The ITFPHT submitted amendments for 2,4-D, silvex and MCPA petitions on December 29, 1970. The Environmental Protection Agency (EPA) acknowledged filing of these amendments December 30, 1970.

(2) The ITFPHT also refiled the 2,4,5-T petition on December 22, 1970 and EPA advised on December 31, 1970 a petition number had been reserved but they (EPA) wished to know which specific 2,4,5-T formulations were to be covered by the petition and for industry to identify the ingredients (active and inert) contained in these products.

(3) The EPA has also advised that a specific tolerance for phenoxies in grass is necessary; a tolerance in grass had not been requested by ITFPHT as it did not appear necessary. In compliance with the EPA request, a level of 300 ppm for all phenoxies in grass was requested on January 6, 1971 by ITFPHT.

(4) On January 11, 1971 Dow was informed that the advisory committee from the National Academy of Science had been appointed on November 2, 1970 to review the food crop cancellation of 2,4,5-T.

At this writing, Dow and Hercules had each been given an hour on February 1, 1971 to present information relative to the safety of 2,4,5-T as used on food crops.

5676

75

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432

Environmental Protection Agency
Chamblee, Georgia 30341

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A Health Survey of Workers in a 2,4-D and 2,4,5-T Plant

With Special Attention to Chloracne, Porphyria Cutanea Tarda, and Psychologic Parameters

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A study of 73 male employees in a 2,4,5-T factory was made. Chloracne was found in 13 (18%) workers. Severity of chloracne correlated significantly with the presence of hyperpigmentation, hirsutism, eye irritation, and a high score on the manic scale of the Minnesota Multiphasic Personality Inventory. Chloracne was not, however, correlated significantly with job location within the plant, duration of employment, or coproporphyrin excretion. Although 11 subjects with uroporphyrinuria and at least three with overt porphyria cutanea tarda had been found in a study of the same plant six years ago, no clinical porphyria could be currently documented and only one worker had persistent uroporphyrinuria. Evidence of toxicity in other organ systems was markedly less than that reported in previous studies and could not be shown to differ from normal populations in most instances.

THE herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) are widely used throughout the world. Several distinct medical problems have been described in workers involved in the production of these compounds. These problems can arbitrarily be subdivided into (1) chloracne and mucous membrane irritation, (2) hepatotoxicity, neuromuscular symptoms, psychologic alterations, and other systemic symptoms, and (3) porphyria cutanea tarda (PCT).

Chloracne and Mucous Membrane Irritation.—Chloracne (perna disease, perchloronaphthalene disease) has been described as an extremely refractory acne seen in workers involved in the production of several chlorinated aromatic compounds.[1-3] Kimmig and Schulz[4-6] described this dermatologic condition in workers in a 2,4,5-T factory. The condition is characterized by inclusion cysts, comedones, and pustules, with eventual scarring, and originates in the temple-zygomatic area with spread to the pinna, nape of the neck, back, upper chest, and inguinal area. Many of these patients also had blepharoconjunctivitis. The investigators showed that purified 2,4,5-trichlorophenol (TCP) was not acnegenic, but that 2,3,6,7-tetrachlorodibenzodioxin (TCDD) isolated from the crude TCP reaction mixture was an extremely potent acnegenic agent when applied to rabbits' ears.[4] The TCDD is con-

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sidered an important, but not necessarily the only, acnegenic compound in 2,4,5-T production.

Hepatotoxicity, Neuromuscular Problems, Psychologic Alterations, and Other Systemic Symptoms.—In addition to chloracne, studies of workers in 2,4,5-T plants have frequently noted other signs and symptoms suggestive of systemic toxicity. These include anorexia and weight loss, abdominal pains and postprandial flatulence, headaches, weakness in the legs, and hepatic dysfunction.[6-10] The following psychological alterations were also noted: lack of vigor, drive, and libido; easy fatigability; emotional instability; and diminished ability to learn.

Porphyria Cutanea Tarda.—Porphyria cutanea tarda is an acquired porphyria, generally occurring in men after age 40, in which significant liver dysfunction and usually a history of alcoholic abuse are found. The syndrome consists of vesiculobullous lesions on areas of the body exposed to light, hirsutism, excessive mechanical fragility of the skin, hyperpigmentation, and excretion of red urine containing an increased amount of uroporphyrin or coproporphyrin, or both, but not a large increase in urinary δ-aminolevulinic acid (ALA) or porphobilinogen (PBG).[11,12] Frequently these patients have hyperferremia and hemosiderosis, and phlebotomy often reverses the clinical picture and decreases porphyrin excretion.[13,14]

In 1964, Bleiberg et al[15] reported studies of 29 workers in a 2,4-D and 2,4,5-T factory. Many of the workers had chloracne and 11 had abnormal excretion of urinary uroporphyrins. Many of the workers with and without uroporphyrinuria had hirsutism, hyperpigmentation, and increased skin fragility. Liver dysfunction was noted in two hospitalized patients.

As a follow-up of the observations of Bleiberg et al[15] of a toxic PCT in these factory workers, we restudied all the employees in the same plant in February 1969 with particular emphasis on PCT, chloracne, hepatotoxicity, and neuropsychiatric symptoms.

Methods

All volunteers submitted to a medical history and physical examination; special emphasis was placed on occupational, smoking, drinking, and medication histories and the detection of any neurologic or dermatologic signs and symptoms. Physical examinations were performed by three different physicians; each physician's findings were considered final and were not confirmed by a second physician.

The Minnesota Multiphasic Personality Inventory (MMPI)[16] was administered to all employees in a quiet atmosphere at work supervised by management. The only exceptions were a few plant administrators who completed the inventory at home. The individual tests were scored by computer, and the mean and standard deviations were computed for each scale and compared with normative data.[16] In addition, the frequencies of high and low two-point scores were determined.

Each employee, after fasting for at least three hours, was given a bottle of a beverage containing 75 gm carbohydrate (Glucola) to drink. Two hours after ingestion, a blood sample was obtained and the serum glucose and other analyses were performed in an automated clinical laboratory. In persons reporting after 135 minutes, the two-hour serum glucose was considered invalid and not included in the statistical analysis. In addition to the glucose determination, serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), alkaline phosphatase, cholesterol, bilirubin, albumin, total protein, blood urea nitrogen (BUN), and hemoglobin values, and an hematocrit reading, red blood cell count, white blood cell count (WBC), serum iron value, and iron-binding capacity were disclosed for each employee.

A routine urine specimen was collected from each volunteer, the pH was adjusted to 7.0, and the specimen was frozen until porphyrin analysis. An attempt was made to shield the urine from ultraviolet light but was not entirely satisfactory in the industrial setting. In addition, each urine sample was analyzed for ALA and PBG by the method of Marver et al[17] and for uroporphyrin and coproporphyrin by the method of Schlenkler et al.[18] The porphyrin and porphyrin precursor levels were expressed per gram of creatinine.

For statistical analysis, employees were subdivided into several different groups according to location in the plant, exposure to chemicals, and educational level. All quantitative results were reported as mean ± one standard deviation, percent of prevalence, or a correlation coefficient between two variables. Significance was determined by Student's *t*-test or χ^2 test; results reported as nonsignificant are those with the significance level (probability value) of greater than 0.05. Where distribution of

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results on a given continuous variable was not normal logarithmic transformations were made before performing *t*-tests or calculating correlation coefficients. Means and standard deviations are always reported as untransformed values.

Results

Descriptive Data.—Seventy-eight persons were studied in the plant. Five women employed in the office were excluded from the analysis. The remaining 73 employees, all men, included one part-time worker and four persons who had not worked in the plant for one to six months. The group of 73 persons was subdivided into four basic occupational subgroups: (1) administrators and technical help, 20 men including engineers, business office workers, laboratory technicians, and janitorial help, all housed in a building separated from the production area and, thus, less exposed to production chemicals; (2) supervisors, 11 foremen spending most of their time in the production area; (3) on-line personnel, 28 persons working in the production area; and (4) maintenance, 14 men with various occupations (plumbers, electricians, etc); some were confined to the maintenance shop and others were frequently in the production area.

As seen in Table 1, the average age of the 18 Negro and 55 white employees was 39.3 ± 11.1 years and the average years of schooling, 11.9 ± 2.6 years. The average duration of employment was 8.3 ± 7.6 years (Table 2). Of 64 workers questioned, 22 missed more than one day from work due to illness in the past year. The average number of days lost for 63 employees was 2.8 days per year. (This excludes one person hospitalized for almost two months with a urinary tract infection.)

Acne.—A specific dermatologic history with special emphasis on acne and porphyria was taken from all the employees and all were examined by one of us (P.P.).

The minimal acne lesion consisted of comedones and inclusion cysts in the temple-zygomatic area and the pinna of the ear. In more severe cases, cysts and comedones were accompanied by pustules on the face and ear, nape of the neck and back, and in the worst cases, the chest and inguinal area. With anatomic spread, the severity of the lesions and the scarring was generally increased. No erythematous or edematous lesions were found, as previously reported by some investigators studying intense or massive exposures.[6]

"Active acne" was defined as the presence of cysts, comedones or pustules (but not scarring). Each of the three lesions was graded according to the severity (grade 0 to 4) in each location (five possible areas: face, neck, back, chest, and other). The severity grades for each lesion were summed over all five locations. Finally, these severity sums were totaled to produce an overall active acne score (maximal possible score: grade 4 in all five locations = 20 for each of three lesions = 60). This arbitrary rating allowed us to deal with the degree of active acne in a statistical form. Scarring was scored separately by the same system (grade 0 to 4 multiplied by five possible areas = maximal score of 20).

Twenty-five persons had an active acne score of 0 (no lesions), 35 had a score of 1 to 8 (minimal), and 13 employees had a score of 9 or greater (moderate to severe). Scarring was also a highly skewed parameter: 37 employees had a score of 0; 29 had a score of 1 to 8; and 7 had a score greater than 9. As seen in Table 3, with increasing active acne scores, the prevalence of scarring, hyperpigmentation, hirsutism, and complaints of eye irritation was greater.

Another way of expressing the same data is to convert the skewed active acne scores to a more normal distribution by logarithmic transformation (see Methods) and compare these transformed scores with other parameters by correlation coefficients. Scarring, also a skewed parameter, was similarly transformed.

Active acne was correlated with the degree of scarring ($r = 0.80$), the presence of hyperpigmentation ($r = 0.38$) and hirsutism ($r = 0.44$), and complaints about eye irritation ($r = 0.39$). All the above correlations have a significance level of $P < 0.001$. The active acne scores were not significantly correlated with the duration of employment nor with the excretion of coproporphyrins per gram of creatinine.

The occurrence of acne was not significantly greater in Negro employees than in white workers (χ^2 test). Although all six

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Table 1.—*Distribution, Age, and Educational Level of Employees According to Job Category*

Group	No. of Employees	Age*	Years of Schooling*
Administrators, lab technicians, janitors	20	34.0 ± 12.2	13.8 ± 2.3
Supervisors	11	46.8 ± 7.4	12.7 ± 3.0
On-line personnel	28	40.1 ± 10.0	10.6 ± 1.7
Maintenance	14	39.1 ± 10.5	10.6 ± 1.8
Total plant	73	39.3 ± 11.1	11.9 ± 2.6

* Mean ± 1 SD.

Table 2.—*Duration of Employment in Plant*

No. of Years*	No. of Employees
0-4	33
4-8	10
9-12	1
> 13	29

* Mean ± 1 SD = 8.3 ± 7.6 years.

individuals who stated that they had had teen-age acne and who had worked in the plant for more than 14 months do presently have active acne, the prevalence of acne in this group (100%) was not statistically higher than the prevalence of acne in a group who had also worked in the plant for more than 14 months but had no history of teen-age acne (62% [$\chi^2 = 3.40$; 1 degree of freedom, $0.05 < P < 0.10$]).

The 73 male employees were subdivided into groups with respect to present work location in the plant: TCP formation, 2,4-dichlorophenol (DCP) formation, phenoxyacetic acid production, esterification, formulation and storage tanks, maintenance, supervisory, laboratory help, and administration. Often one man might work in several locations (eg, supervisors might work in both TCP and DCP production and in phenoxyacetic acid production and esterification). In these cases, each area was compared (χ^2 test) to see if the severity of the active acne of the employees might be worse in certain locations in the plant. There were no statistically significant differences, perhaps because of the small size of each group and the mobility of the workers. However, as might be expected, the maintenance men tended to have the most acne (having to clean and repair vats and pipelines) and the administrative people (housed in a separate building) tended to have the least acne.

Mucous Membrane Irritation.—Seven employees complained of itching of the eyes, 14 of frequent tearing, five of "bloodshot eyes," and seven of sties. On physical examination, however, only three (4.1%) had appreciable conjunctival injection. Twenty-three (31.5%) employees had hyperemia of the nasal mucosa and eight (10.9%) had inflammation of the buccal mucosa.

Hyperpigmentation and Hirsutism.—Hyperpigmentation, usually most prominent on the face, consisted of a grayish or brownish tone to the complexion. Hirsutism was most noticeable in the area between the outer edge of the eyebrow and the temple hair margin. Both characteristics were recorded only as being present or absent. As seen in Table 3, 16 subjects had facial hypertrichosis and 30 had hyperpigmentation. Correlation between hirsutism and increased hyperpigmentation was significant ($r = 0.25$, $P < 0.05$). As previously mentioned, hirsutism and hyperpigmentation are significantly correlated with the severity of active acne, but neither is significantly correlated with coproporphyrin excretion.

Urinary Excretion of Porphyrins and Porphyrin Precursors.—The average values of ALA, PBG, and coproporphyrin found in routine urine specimens of 72 workers in the plant are shown in Table 4 along with the normal values from the literature.[19] The excretions of ALA, PBG, or coproporphyrin were not abnormally elevated. One employee, hospitalized several years ago with severe PCT, still has mild uroporphyrinuria (107 μg/gm of creatinine). However, no overt clinical cases of porphyria were found during this study. Only four of the 11 workers with uroporphyrinuria originally described by Bleiberg et al[15] were still working in the plant. Several of the seven employees with uroporphyrinuria who had left the plant had been retested by Dr. Bleiberg before they left and had no elevation in urinary porphyrin excretion.

Several pertinent observations can be made about these urinary porphyrin values. The excretion of ALA and PBG, although not abnormally elevated, nonetheless tended to be relatively increased in the 15 maintenance men versus the other 56 workers. The coproporphyrin excretion per gram of creatinine, although again within normal limits,

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was significantly elevated in the maintenance men versus the same 56 workers (48.3 ± 14.4 vs 36.7 ± 19.6, $P < 0.025$). Persons with higher values for coproporphyrin excretion (≧ 50 mg/gm of creatinine, N = 15) did not have any greater duration of employment in the plant than those with lower values (χ^2 test). There was a significant positive correlation between ALA excretion and coproporphyrin excretion ($r = 0.42$, $P < 0.001$) and between ALA excretion and age ($r = 0.42$, $P < 0.001$). There was a significant negative correlation between ALA excretion and serum bilirubin concentration ($r = -0.26$, $P < 0.05$).

Cardiovascular Findings.—Pulse rate and blood pressure recordings were taken. The average pulse was 85.1 ± 10.2 beats per minute; systolic blood pressure, 126.6 ± 12.8 mm Hg; and diastolic blood pressure, 75.4 ± 7.8 mm Hg. Only four persons had a diastolic pressure greater than 90 mm Hg. Cardiac evaluation was unremarkable except for histories of myocardial infarction in three employees.

Pulmonary Findings.—Twenty of 73 employees (27.4%) gave a history of a chronic cough, emphysema, or fairly consistent raising of early morning sputum. Of these 20 workers, 16 (80%) currently smoke cigarettes and the other four (20%) had smoked considerably in the past. In the entire plant, 42 employees (57.5%) currently smoke cigarettes and another 15 (20.6%) gave a history of smoking in the past. The group of current smokers averages 25.6 pack-years.

On physical examination 18 workers were judged to have some impairment of maximal diaphragmatic excursion. Fourteen of these currently smoke and three had smoked in the past. Eight of these 18 workers gave a history of chronic cough or frequent early morning sputum and one gave a history of asthma.

Although the incidence of chronic pulmonary disease in the plant seems to be appreciable, it cannot be attributed to any industrially related cause, as suggested by the report of Bauer[7] because 78% of the employees now smoke or have smoked cigarettes previously.

Hepatic Function.—All serum total bilirubin values and albumin concentrations were within the normal laboratory range (Table 5 includes mean values). Two subjects had alkaline phosphatase values slightly above the normal laboratory range (18.7 and 19.0 King-Armstrong units/100 cc, respectively [normal range, 7 to 18 King-Armstrong units/100 cc]); the other liver function tests were normal. Two employees had SGOT levels elevated above the normal range (48 and 121 Karmen units/ml, respectively [normal range, 7 to 45 Karmen units/ml]), but otherwise had normal liver function tests. The employee with an SGOT value of 48 Karmen units/ml was receiving lincomycin injections at two- to four-week intervals. The SGOT value of 121 Karmen units/ml was not confirmed by a second testing, and the subject had no other evidence of liver malfunction. Six persons had palpable liver edges; five of these six findings were observed by one of the three physicians. Two of these six subjects were heavy beer drinkers (at least four cans of beer per day), and one had emphysematous lung changes with lowered diaphragm. Only one of the six subjects with a palpable liver had an abnormal liver function test, an alkaline phosphatase level of 19 King-Armstrong units/100 cc.

Gastrointestinal Results.—Several studies of 2,4,5-T workers have mentioned high prevalences of anorexia and weight loss, postprandial flatulence, nausea and vomiting, abdominal pain, and documented cases of gastritis and ulcers.[4,6-8,10] At the time of the examination 22 workers in this plant (30%) were intermittently experiencing at least one of the following symptoms: nausea, vomiting, diarrhea, abdominal pains, or blood in the stool. Two persons in this group and four other persons had a history of "ulcers" (6 of 73 persons or 8.2%). We found no results from other industrial settings with which to compare our data.

Neurologic Findings.—Several authors have noted lower extremity weakness and fatigue in many of the patients with chloracne in 2,4,5-T factories.[1,7] Only seven of 73 (9.6%) employees in our study complained of lower extremity fatigue or difficulty in climbing stairs. Five of these workers gave a history of arthritis in the lower extremities or heart disease with dyspnea; thus, only two (2.7%) experienced leg fatigue totally unexplained by other concomitant disease. Lower extremity weakness was not detected

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in the neurologic examination of any subject.

Eight workers (11%) complained of nonspecific headaches. Ten workers had decreased auditory acuity (inability to hear a watch ticking 1 cm from the ear); four of these had tympanic membrane disease. No discrimination was made between nerve or bone conduction defects. A decrease in olfactory discrimination was observed in only one employee and was most likely due to nasal obstruction. Two persons had a decreased sense of proprioception (one with history of chronic lumbar arthralgia secondary to traumatic injury to the back). In three persons, Achilles tendon reflexes were absent without other neurologic deficits (one had a two-year history of diabetes mellitus). Tremors of the hands were observed in three employees. We found no abnormalities of cranial nerves other than those noted above: no clonus, no alterations in deep tendon reflexes of musculi biceps or triceps brachii or quadriceps femoris, and no alteration in pain sensation.

Table 3.—*Distribution of Certain Signs, Symptoms, and Test Scores Among Three Categories of Active Acne Severity*

	Active Acne Score		
	0	1-8	≥9
No. of employees			
Hirsutism	3/25 (12%)	4/35 (11%)	9/13 (69%)
Hyperpigmentation	5/25 (20%)	15/34 (44%)	10/12 (83%)
Scarring	1/25 (4.0%)	22/35 (63%)	13/13 (100%)
Eye irritation (history of tearing, itching, sties, bloodshot)	1/25 (4.0%)	8/35 (23%)	6/13 (46%)
Mean score of Mania scale of MMPI	55.4 ± 7.7	58.8 ± 10.2	64.9 ± 10.5*

* The persons with acne scores of > 9 had significantly higher scores on the Mania scale on the MMPI ($P < 0.05$).

Results of Other Laboratory Determinations.—Only one employee had an elevated BUN value, as shown in Table 5 (29 mg/100 ml; normal range, 6 to 24 mg/100 ml), and none had an elevated uric acid level (normal range, 3.0 to 7.9 mg/100 ml). The serum cholesterol (normal range, 133 to 294 mg/100 ml) was elevated in seven of 71 persons. There was an unexplainable elevation in the serum LDH (normal range, 50 to 160 Wacker units/ml) in 20 of 70 employees (28.6%); this may have been due to centrifuging clinical chemistry blood specimens more than two hours after sampling.

The two-hour serum glucose levels, as determined by the automated ferricyanide method for glucose determination, were judged satisfactory with 58 employees (see Methods). Six employees (10.4%) had a two-hour serum glucose value of ≥ 140 mg/100 ml[20] after a 75-gm oral carbohydrate load (145, 148, 152, 171, 174, and 176 mg per 100 ml). The average age of these six individuals (50.6 years; range, 39 to 62) was higher than that of the 52 individuals whose results were < 140 mg/100 ml (39.3 years; range, 21 to 56). One of the six was the only known diabetic in the plant; he had a 19-year work history in the plant and a two-year history of diabetes which is currently being treated with tolbutamide. The average period of employment in this plant for the six individuals was 9.2 years, with the range being 1 to 19 years; three had minimal chloracne and one had severe chloracne. None of the six had a family history of diabetes; one was obese. The prevalence of 10.3% hyperglycemic results in this factory is consistent with a 14% prevalence figure of two-hour postglucose hyperglycemia in a random sample of a normal population in Bedford, England.[21]

Only two of 72 employees had a hemoglobin value of less than 13 gm/100 ml of blood, and 16 employees (22%) had a serum iron determination of less than 75μg/100 ml. The mean serum iron value was 97 ± 32μg/100 ml and only two employees had a value of 160μg/100 ml or greater. The average value for saturation of the iron-binding capacity was 27.4% ± 9.0%, with 27 persons (37%) having a value of less than 25%. Seven persons had a WBC of less than 5,000 cells/cu mm, but it was 4,000 cells/cu mm or less in only two employees. None of the seven had an absolute granulocytopenia (< 1,500 neutrophils/cu mm)[22] and only one worker had a lymphopenia (< 1,000 lymphocytes/cu mm).[22] Since the normal hematologic values used here are the 95% confidence limits of a study of a normal population,[22] single cases of granulocy-

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Table 4.—*Urinary Porphyrin Excretion*

Test	Plant Values, μg/gm of Creatinine*	Normal Values[19]	Upper Limit of Normal, μg/gm of Creatinine
ALA	1,021 ± 406	1,900 ± 600 μg/gm creatinine	3,100
PBG	444 ± 237	700 ± 400 μg/gm creatinine	1,500
Coproporphyrin	39.3 ± 19.0	0-175 μg/liter	175‡
Uroporphyrin	Trace†	0-15 μg/liter	15‡

* Mean ± SD, N = 72.
† Measurable in only one person; value, 107μg/gm of creatinine.
‡ Values derived from normal by assuming a 70-kg man excretes creatinine at ≥ 1.5 gm/day and 1,500 ml of urine.

topenia and lymphopenia would not be unexpected in a population of 73. The red cell morphology and platelet count, determined by scan of the peripheral blood smear, were normal in all subjects.

MMPI Results.—The MMPI scores of production workers (including on-line personnel, supervisors, and maintenance men [N = 52]) and the administrative staff (including laboratory technicians [N = 17]) were analyzed separately because of the difference between the two groups in environmental exposure and educational level. Four janitors, currently working in the administration building, were eliminated from the analysis of the administrative staff because of a different educational background and previous exposure as production workers.

MMPI results are based on an analysis of scores of all subjects on 13 separate scales. Ten of these scales are designated by common clinical terms and are thus somewhat self-explanatory (Table 6). Detailed descriptions of each scale are found in *An MMPI Handbook*.[16] The remaining three scales, the L, F, and K scales, measure the validity of the test results as an accurate profile of the subject. A high L score indicates that the subject answered most questions according to socially desirable standards rather than according to individual preferences. More highly educated subjects tend to have lower L scores; this is consistent with our finding of significantly lower L scores in the administrative staff. A high F score may be obtained if a subject answers in a prankish way, if he has a poor ability to read or comprehend the items, or if he is severely mentally disturbed. Since only one subject out of 69 had a high F score (F > 76), the test seems to have been both understood and taken seriously by the workers. A high K score indicates a high degree of defensiveness in a person (an unwillingness to admit that he differs in any respect from what he considers normal); this score increases with educational level. The mean K scores for both groups in this plant are normal for their educational levels and do not significantly differ from each other.

The only significant differences in means on all scales between administrative staff and production workers were on the L and Hypochondriasis scales, with the production workers presenting a more hypochondriacal picture than the administrative staff. When scores of both groups of employees were compared with the normal values given for the MMPI test scales (N, approximately 300; mean, 50; standard deviation, 10), administrative staff and production workers varied significantly from normal on a total of six and nine scales, respectively (Table 6). The normal values for the MMPI, however, were based on scores of a small population of Minnesota males who took the test just before World War II.[16] Since many obvious demographic differences exist between the plant population and the "normal population," it was thought that a more meaningful definition of the plant population would be obtained by searching for personality patterns appearing in high frequencies within the plant. A high-point code system was used which grouped subjects according to the two scales in which they scored the highest. The following high-point codes had the greatest frequency (F): administrative staff, hypomanic-hysterical, f = 23%; production workers, hypomanic-psychopathic, f = 12%. It is apparent from these small frequencies that no one personality pattern tended to dominate either administrative or productive group.

Correlation coefficients were calculated

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for several MMPI scales with other parameters, such as porphyrin excretion, chloracne, job history, and several biochemical tests. A significant correlation was found between the score of severity of active acne and the hypomanic scale ($r = 0.33$, $P < 0.01$). Furthermore, the mean manic scale score for the group with the most severe chloracne was significantly higher than the means of the two groups with less severe acne ($P < 0.05$ [Table 3]).

Table 5.—*Clinical Laboratory Data*

Test	Mean	SD	N*
BUN (mg/100 ml)	14.5	3.9	72
Uric acid (mg/100 ml)	5.4	1.0	72
Cholesterol (mg/100 ml)	237	44	71
Two-hour serum glucose (mg/100 ml)	95	32	58‡
Alkaline phosphatase (King-Armstrong units)	11.2	2.5	71
LDH (Wacker units)	146†	46	70
SGOT (Karmen units)	22	13	71
Bilirubin (mg/100 ml)	0.44	0.16	71
Albumin (gm/100 ml)	4.6	0.3	71
Hemoglobin (gm/100 ml)	14.9	1.1	72
Serum iron (μg/100 ml)	97	32	70
Total iron-binding capacity (μg/100 ml)	348	44	69
Saturation of iron-binding capacity (%)	27.4	9.0	69
WBC (cells/cu mm)	7,160	2,190	72

* Variation in N secondary to sampling difficulties; for N = 73, mean age ± SD = 39.3 ± 11.1; 25% of population are Negroes, 75% are white.
† For N = 58, mean age ± SD = 39.3 ± 10.9; 29% of population are Negroes, 71% are white.
‡ Elevated value is probably due to poor sample handling.

Comment

Acne.—Fourty-eight of 73 (66%) employees in our study had some degree of acne, but only 13 (18%) had moderate to severe lesions. Most previous investigations of acne occurring in workers in 2,4,5-T plants have selectively presented clinical and pathologic findings in those severely affected. In contrast, the present study surveyed the prevalence of acne in the entire employee population and found the acne absent or minimal in 82% of the workers.

The severity of active acne correlated with the presence of scarring, hyperpigmentation, hirsutism and complaints about eye irritation (Table 3). Dugois et al[10] reported a 50% incidence of conjunctivitis in 2,4,5-T workers with acne. Although we had many complaints about eye irritation, only three workers were observed to have conjunctivitis. However, 23 (32%) of the employees had hyperemia of the nasal mucosa and eight (11%) had hyperemia of the buccal mucosa.

Our findings that the presence of hyperpigmentation and hirsutism correlate quite significantly with the severity of acne and not at all with coproporphyrin excretion suggests that these two signs, which are usually thought of as part of the PCT syndrome, may be more closely related to chloracne in 2,4,5-T plants. Other studies support this view, such as (1) the findings of Bleiberg et al[15] that the presence of hirsutism and hyperpigmentation correlated better with the presence of chloracne than with uroporphyrinuria, and (2) the findings of other studies of 2,4,5-T factories[7,23] in which workers were noted to have chloracne and hyperpigmentation but no mention was made of skin fragility, vesicular eruptions, or red urine.

As stated in the introduction, Kimmig and Schulz[1-6] have demonstrated the potent acnegenic properties of TCDD, an unwanted side product in the synthesis of 2,4,5-T. There is a tendency in the plant we studied for the maintenance men, who have the most direct exposure to all products of synthesis, to have the most severe acne and for the administrative personnel, who have the least exposure, to have the least acne. However, an association of a higher prevalence of acne with any particular location in the plant could not be demonstrated, eg, in the TCP synthetic area where one would expect TCDD concentration in the environment to be the highest. The plant has taken steps to decrease the contamination of the TCP end product by dioxin, and the concentration of the contaminant in the TCP has dropped from 10 to 25 ppm to 1 ppm (see the following subsections on chemistry and the plant). At the time of the study the effect of the safety measure to decrease contamination on the severity or prevalence of chloracne in the factory could not be evaluated since (1) there were no previous prevalence

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Table 6.—*Scale Scores on the MMPI*

	52 Production Workers			17 Administration Workers		
Scale	Mean	SD	P*	Mean	SD	P*
L	52.5	8.2	NS	46.1	5.8	NS
F†	54.6	7.4	<0.0025	54.4	8.8	NS
K	53.2	8.9	<0.025	55.4	6.3	<0.025
Hypochondriasis‡	55.5	9.8	<0.00025	48.8	8.5	NS
Depression	59.5	11.2	<0.0001	56.7	11.5	<0.01
Hysteria	59.0	8.8	<0.0001	58.9	6.7	<0.001
Psychodeviance	59.8	12.1	<0.0001	55.5	12.1	<0.025
Masculinity	57.4	7.6	<0.0001	60.7	6.1	<0.00025
Paranoia	52.9	8.3	<0.05	52.6	9.6	NS
Psychasthenia	52.1	10.4	NS	50.8	8.9	NS
Schizophrenia	51.2	9.8	NS	51.2	10.0	NS
Mania	60.0	9.0	<0.0001	56.8	12.4	<0.01
Social introversion	51.4	8.4	NS	50.1	9.8	NS
Years of schooling	10.9 ± 2.1			15.2 ± 1.5		P 0.001§
Coproporphyrin excretion, μg/gm of creatinine	38.8 ± 16.0			37.9 ± 24.7		NS§
No. of persons with some degree of acne	36/52			10/17		NS‖
Active acne score	6.2 ± 9.6			2.5 ± 3.2		NS§

* Significance values by Student's t-test, comparing the above populations to the normal population as defined in the text of the results.
† The two plant groups are significantly different ($P < 0.005$). This would be expected on an education basis.
‡ The two plant groups are significantly different ($P < 0.01$).
§ Significance by Student's t-test.
‖ Significance by X^2 test.

figures on chloracne in the plant with which to compare and (2) the effect of such a measure on the severity or prevalence of a chronic disease such as chloracne may take longer than six months to become evident.

Individuals seemed to vary greatly in their susceptibility to acquire chloracne. This observation is consistent with (1) the lack of correlation between the duration of employment in the plant and the severity or presence of chloracne and (2) the tendency toward an increased susceptibility to chloracne in persons with history of teen-age acne.

Chemistry.—Although the structures of 2,4-D and 2,4,5-T (Figure) differ only by one chlorine, the commercial syntheses of the two compounds are dissimilar. The starting product for 2,4,5-T is 1,2,4,5-tetrachlorobenzene which reacts with methanol and aqueous sodium hydroxide to form 2,4,5-trichlorophenate, 2,4,5-trichloroanisole, and small amounts of several unwanted side products, eg, TCDD. The reaction product is steam-stripped to remove the anisole. At this point, sampling of various batches shows the TCDD to be present in 10 to 25 ppm. About six months before our survey, the company installed a device which removed most of the TCDD. The TCP thus produced contained less than 1 ppm TCDD.

The TCP reacts with monochloroacetic acid (MCA) to form 2,4,5-T. The latter is esterified with various alcohols or allowed to react with dimethylamine, and then formulated and packaged. Some 2,4,5-T and 2,4-D is sold as the unesterified acid in solid form.

The starting materials for 2,4-D are phenol and chlorine (gas) which react in an exothermic process to form DCP. Subsequent reactions with MCA to the phenoxyacetic acid are similar to 2,4,5-T. In 2,4-D production there is no formation of the TCDD.

The Plant.—The plant employs several measures in an attempt to improve industrial hygiene. Clean work clothes are issued daily, and separate lockers for street and work clothes are provided. All production workers said they showered daily before leaving the plant. The DCP and TCP are made in one building and the 2,4-D and 2,4,5-T (acids) and their esters in another. Attempts have been made to improve ventilation, and recently the company has been successful in removing most of the TCDD before the TCP leaves the area in which it is synthesized. A dermatologist employed by the company visits the plant at least weekly, largely to treat the chloracne.

Almost all operations are carried out in closed processes (eg, tank car to pipeline to reaction vessel to next pipeline to next reaction vessel). There is of course some leakage, and frequently men working in maintenance must clean the inside of the reaction tanks or open pipelines.

Porphyria Cutanea Tarda.—A large outbreak of PCT in Turkey in 1956 due to the accidental ingestion of hexachlorobenzene[21-25]

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plus the high prevalence of PCT described by Bleiberg et al[15] in this 2,4-D and 2,4,5-T plant in 1964 support a direct toxic etiology of many cases of PCT. The actual etiologic agent of PCT in this plant is unknown. We have administered reagent grade DCP to rats and observed an increase in the urinary excretion of coproporphyrin (unpublished data). A single oral dose of DCP caused a threefold increase in the activity of hepatic ALA synthetase, the rate-limiting enzyme in porphyrin biosynthesis. Rimington and Ziegler[29] reported a series of structurally similar chlorinated benzene compounds which caused experimental porphyria in rats. The TCDD, because it is a potent hepatotoxin and acnegen, was not examined for its ability to cause porphyrinuria in experimental animals.

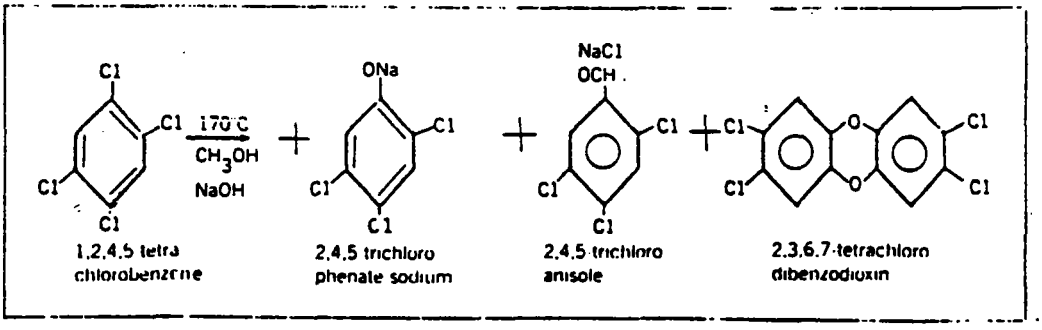
In the report of Bleiberg et al[15] in 1964 of this same plant, 11 employees had uroporphyrinuria and several had hirsutism, hyperpigmentation, or skin fragility, or all three. In the three case histories presented, two patients had vesicular eruptions on exposed areas of the body, two had hepatic dysfunction, and the tissue obtained from the two reported liver biopsies fluoresced intensely with ultraviolet irradiation. These three case histories unquestionably fulfilled the criteria for the diagnosis of PCT.

Our reexamination of the same plant six years later revealed no clinical PCT; only one employee had mild persistent uroporphyrinuria. The reason for the decrease in severity and prevalence of PCT in this plant is unknown. Possible reasons include (1) increased encouragement of personal safety habits of workers, and (2) decrease in the contamination of PCT by the hepatotoxin, TCDD, and perhaps simultaneously by porphyriagenic chemicals.

Porphyria cutanea tarda (ie, uroporphyrinuria, skin fragility, and vesicular eruptions on the skin) does appear to be a symptom-complex distinct from that of chloracne (ie, cysts, pustules, comedones, and scarring of the skin); both were found in workers in this plant in which 2,4-D and 2,4,5-T are manufactured. The independence of these syndromes is supported by (1) the lack of correlation between the severity or even presence of chloracne and uroporphyrinuria in the previous study,[15] and (2) the persistence of chloracne in the factory six years later, despite the disappearance of the uroporphyrinuria and skin fragility. Even if the etiologic agent is the same, the syndromes apparently can occur independently and certainly have different clinical courses.

The statistically higher coproporphyrin excretion in the maintenance men suggests that they have a higher toxic exposure; they also have a tendency towards more severe acne. These men repair the inside of reaction vessels and leaking pipelines, and although their exposure is sporadic, it is apparently more intense.

Recently, much attention has been directed towards the finding of hemosiderosis and hyperferremia in patients with PCT, often in those with an alcoholic history.[30-32] Although studies of iron metabolism were not reported in the patients with hexachlorobenzene intoxication, chelating agents did appear to reverse the clinical symptoms and porphyrin excretion.[33] In our study, despite the absence of uroporphyrinuria in the workers at the present time, a tendency towards hyperferremia might be expected. On the contrary, we found an unexplainable trend towards hypoferremia and a lower iron-binding saturation, with little demonstrable anemia.



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Hepatotoxicity.—TCDD, which has been incriminated as a potent acnegenic agent, is also very hepatotoxic. A single oral dose of 0.05 to 0.1 mg/kg to rabbits resulted in death in one to two weeks. Autopsy revealed extensive necrosis and enlargement of the livers.[5] Similarly, dermal application of the tarry residues in 2,4,5-T synthesis caused gross hepatic lesions and rapid death in guinea pigs.[10] Clinically, several investigators have looked for evidence of hepatotoxicity in 2,4,5-T-plant workers affected with chloracne. Bauer et al[7] noted that two of nine cases had slightly delayed sulfobromophthalein excretion and three had abnormal liver biopsies. In the report of Bleiberg et al[15] of patients with chloracne and porphyria, two subjects had hepatic dysfunction and two had abnormal liver biopsies. Dugois and co-workers[8,10] have noted hyperlipemic and hypercholesterolemic sera in their subjects. Hofmann and Meneghini[23] found no hepatic damage in six patients with chloracne. Of the liver tests performed in this study (bilirubin, albumin, SGOT, and alkaline phosphatase), only four values were elevated. Three of these four elevations were extremely mild, and the fourth was accompanied by no other abnormal liver test values. Of the six patients with palpable livers, only one had a mildly elevated liver function test. Although TCDD and other chemicals produced in 2,4,5-T synthesis may be hepatotoxic in humans, the prevalence of demonstrable chemical liver dysfunction in this plant is minimal.

Systemic Toxicity.—As noted in the introduction, a variety of other signs, symptoms, and laboratory findings have been noted in various studies of 2,4,5-T workers with acne. We found no unexplainable significant increases in abnormal function of cardiovascular, pulmonary, intermediary metabolic, or hematologic systems in comparison with normal populations. Although the prevalence of gastrointestinal complaints seems high (30%) and although physical examination revealed occasional unexplainable neurologic deficits (decreased hearing, six of 73 subjects; diminished proprioception, one of 73; absent ankle jerks, two of 73), we could find no figures of comparable studies with which to compare; hence, a judgment on the normality or abnormality of the prevalence of these findings in this population would only be conjectural.

MMPI.—The MMPI results are important in that they offer an objective profile of the workers in one herbicide synthesizing plant. Conclusions about personality changes secondary to chronic exposure in a plant in which 2,4-D and 2,4,5-T are manufactured can only be tenuous, however, because we have no suitable group with which to compare these results. The comparison of MMPI results of these factory workers with the normal population of pre-World War II, white subjects from Minnesota was inescapable since the scoring system for the test is based upon this "norm."[16] Nonetheless, we consider these groups incomparable. The only evidence to suggest any environmental effect of this herbicide plant on personality is the significant correlation between severity of chloracne (a known toxic effect) and the score on the hypomanic scale. This is an interesting finding and somewhat contradictory to the apathy and psychomotor retardation reported by Bauer et al.[7]

Many previous investigations of both acute and chronic poisoning in 2,4,5-T plants have reported the symptoms of anorexia with weight loss, fatigue, debility, and asthenia.[4,7] However, only one author has described in detail personality changes thought to be associated with chemical exposure. Bauer et al[7] studied nine workers with severe chloracne which still persisted five years after termination of their exposure in a 2,4,5-T plant and described a syndrome of general weakness, fatigue, apathy, and decreased libido alternating with fits of anger and irritability. Rorschach tests showed a weakened emotional reaction, a lack of concentration, slowed thought processes, and perseveration. This whole syndrome was designated by the term "psychovegetative disorder."

The inconsistency of our finding of a significant correlation between hypomania and chloracne and the previous description of lethargy, dulled emotional response, and apathy[7] remains to be adequately explained. Possible reasons include a basic difference in methodologies and a difference in testing period with relation to the time of exposure. Additionally, the severity and chronicity of the organic disease in subjects studied by

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Bauer et al[7] may have induced personality changes which masked any manic effects found with less severe disease.

Thomas Ashby, MD, Augustin Gombart, MD, Jerome Schulz, MD, and Arthur DePalma, MD, provided clinical assistance. Patricia Hickman performed the ALA, PBG, and porphyrin determinations. Janet Daling, MS, Community Study on Pesticides, Washington State Department of Health, assisted in preparing a subset of our data for computer analysis. Frank Clark, PhD, Assistant Professor of Psychiatry (Psychology) at Emory University, assisted in interpretation of MMPI results.

Jacob Bleiberg, MD, gave permission for entry of the factory studied and provided written records of past medical histories for comparison on some workers.

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NEW WORLD

PSAC Hiccoughs Over 2,4,5-T

by our Washington Correspondent

In October 1969, the President's science adviser, Dr Lee Dubridge, set up a panel to study the widely used herbicide 2,4,5-T and its suspected teratogenic properties. The panel duly reviewed the available evidence but, as is customary with reports compiled for the President's Science Advisory Committee, its findings were not made public. The report of the PSAC panel would probably be of little more than historical interest had it not recently been discovered and issued this month as a model of what such a report should be. "This review represents an outstanding case study of government regulation of pesticide," states a press release issued by the Office of Science and Technology. In a preface to the report Edward E. David, who succeeded Dubridge as science adviser and chairman of PSAC last August, says that the report is "an excellent case study" and that the principles it brings out "will be useful in dealing with other pesticides, and indeed with chemicals of all kinds." How far does the review live up to the claims made for it?

Some people who have seen the report call it excellent, others say it contains glaring and serious omissions. The verdict may vary according to the perspective of the beholder and the date and purpose for which the review was compiled. Members of the PSAC panel say it was written so long ago—about March or April 1970—that they can remember little about it. One member believes that, to begin with, the only real data available were the study conducted by the Bionetics Corporation which first showed that commercial preparations of 2,4,5-T could produce malformations in the offspring of rats.

Although the PSAC panel was told to concentrate on the domestic use of the herbicide, this member recalls there was the feeling that evaluation of the military use of 2,4,5-T as a defoliant in Vietnam was the real impetus for undertaking the study. Dubridge, in the first public reference to the Bionetics study in October 1969, said that the Defense Department in its defoliation operations over Vietnam "will restrict the use of 2,4,5-T to areas remote from the population", but for another six months he was unable to stop either the Defense Department or the Department of Agriculture from exposing the populations under their respective control to 2,4,5-T. It was not until April 1970, shortly after the PSAC panel apparently completed its report and confirmed that the Bionetics results could not be dismissed, that the Pentagon's long attachment to 2,4,5-T was broken. On April 15 last year the Surgeon General announced that 2,4,5-T would be suspended for use over water, around the home and on food crops, enabling the Defense Department to declare at the same time that the military use of the herbicide would fall in line with domestic use.

If the purpose of the PSAC panel on 2,4,5-T was simply to strengthen Dubridge's hand by providing an authoritative review of the then available evidence, the panel members seem to have performed a thorough and careful task. But their report, which does not seem to have changed significantly since they completed it, is being presented by the Office of Science and Technology as an outstanding case study of government regulation, which for all intents and purposes covers the evidence on 2,4,5-T up until March 1971, the date of David's preface.

Judged in this light, the PSAC report has several curious features. One is the inclusion of a section on the importance of 2,4,5-T as a defoliating agent. This is an issue which might properly be regarded as beyond the competence of a scientific review panel; if it were to be considered, an impartial presentation of evidence might be expected. The PSAC report is not impartial—it quotes at length, though admittedly without endorsement, a series of anecdotes told by an Admiral W. E. Lemos indicating that defoliation and crop destruction by herbicides are militarily effective. A staff member of the OST has said that this was the only sort of evidence available. In fact there exist several studies made in or for the Department of Defense that challenge the effectiveness of defoliation and crop destruction programmes, including, for example, a survey compiled by the Rand Corporation in 1967 suggesting that only 5 per cent of enemy soldiers depended on locally grown crops.

Why did the PSAC panel fail to cite these reports or to present the evidence objectively? "The reason PSAC did it was a sheer tribute to the political power wielded by the Joint Chiefs of Staff," says one observer. "It was not the habit of PSAC to buck the Joint Chiefs of Staff, at least not under Dubridge." Whatever the reason, the abandonment of scientific objectivity in a kow-tow to the military is an awkward adornment of what purports to be an "outstanding case study of government regulation of pesticide", since if partisan evidence from the Defense Department can be quoted without challenge how is the reader to be sure that similar courtesies have not been extended to the manufacturers of a pesticide and their customers?

Another anxiety raised by the PSAC study lies in its treatment of the economic consequences of 2,4,5-T. The weighing of economic benefits against the possible health hazards of a pesticide is of course a crucial question for regulatory authorities and one that an "outstanding case study" might well be expected to address. But although the panel attempts to estimate the costs of substituting alternative herbicides to 2,4,5-T for a variety of uses, the report contains no suggestions as to how these should be weighed against the toxicological risks.

This failure to grapple with an important issue is in a way not surprising since the necessary expertise in social science is lacking. None of the members of the PSAC panel seems to be an economist, there are only three social scientists on PSAC, and the Office of Science and Technology has yet to appoint its first social scientist staff member. This composition of course reflects the long-standing dominance of physical scientists on PSAC.

Even when on home territory, the PSAC panel report does not seem to have covered all the ground. "For what it does say, it is a reasonably good report, but there are two important recommendations that should have been made but were not," says Matthew S. Meselson, professor of biology at Harvard University. Meselson believes the report should have been more circumspect in its consideration of the dose-response relationship between 2,4,5-T and the terata it induces, and that more attention should have been devoted to the possibility that dioxin, the highly lethal contaminant of 2,4,5-T preparations, may accumulate in body tissues. In essence, the PSAC panel report says that there exists a minimum dose below which the herbicide will not cause damage, so that animals and humans may safely be exposed to concentrations of 2,4,5-T that are small by a safety factor of 10 to 100 than this minimum or threshold dose.

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Meselson agrees that the idea of a threshold is plausible, though unproved, but he believes the PSAC panel report should have pointed out the crucial nature of this assumption, and that in its recommendations for further research it should explicitly have cited the need for establishing a dose-response curve for the teratogenicity of 2,4,5-T. The consequences of the response being linear would be profound.

Meselson also considers the report should have mentioned the possibility that the dioxin contaminant of 2,4,5-T may accumulate in the body fat, as might be expected from its chemical structure. Edward J. Burger, an OST staff member of the panel, says this issue was discussed in panel meetings but not in the report since it was just one among several unproved possibilities. But the possibility that an extraordinarily toxic contaminant of a widely used herbicide (the LD_{50} of dioxin is 0.0006 parts per million for female guinea-pigs) may be sufficiently stable in the environment and soluble in fat and liver to enter food chains and the human diet does seem worth mentioning in what is claimed to be a model report. Moreover, the PSAC panel report offers no scientific justification for its recommendation that the level of dioxin in 2,4,5-T should be limited to not more than 0.5 ppm and should be reduced to 0.1 ppm. This is good news for manufacturers because, as the PSAC panel report points out, present technology allows 2,4,5-T to be manufactured with less than 1 ppm of dioxin.

But the smallest quantity of lethal impurity a manufacturer is capable of admitting into his product is not necessarily the quantity guaranteed to be harmless to man and beast. A very rough calculation indicates that even the 0.1 ppm limit urged by the PSAC panel report raises questions. Animals are quite often found to contain in their bodies concentrations of DDT of the order of 10 ppm; if the pesticide were not DDT but a solution of 2,4,5-T containing 0.1 ppm of dioxin, the animal would contain 10^{-12} parts of dioxin, which is only a thousand times less than the LD_{50} for guinea-pigs (2,4,5-T does not accumulate, but dioxin may). The PSAC panel report includes no calculations of this kind, much less any discussion of their significance.

Of wider interest than the issue of 2,4,5-T, the PSAC panel recommends that new information on a pesticide "should be disseminated promptly to individuals and organizations that are legitimately concerned". The original experiments on 2,4,5-T by the Bionetics Corporation, under contract to the National Cancer Institute, were completed about August 1968, and it was not until 14 months later, after extensive prodding by individuals inside and outside the government, that the existence of the report was first made known by Dubridge.

The PSAC panel report does not deem it worth describing this extraordinary chain of events in any detail. Another recommendation of general importance—now embodied in an administration bill before the Congress—is that a legal mechanism should be established for restricting a pesticide temporarily on the basis of new information that indicates it may be hazardous. Of the two mechanisms available at present, cancellation is too mild—the manufacturer can continue to sell the substance pending an appeal—and suspension is too drastic, since a compound can only be suspended if shown to be an imminent hazard, and once suspended is unlikely ever to return to the market.

A third recommendation is a level-headed warning on the interpretation of teratogenesis. Most chemicals might be shown to be teratogenic in animals, the PSAC report says, and it is not possible to extrapolate the results of animal experiments directly to man. "To restrict or ban usage of chemicals on the basis of demonstration of teratogenicity at dose levels which far exceed actual or expected exposures is unreasonable and could well deny the usage of chemicals whose benefits far outweigh the risks."

The President's Science Advisory Committee won its spurs as a body of physical scientists, concerned almost exclusively with defence matters, whose particular *forte* was the ability to take on issues such as strategic weapons that cut across different parts of the government. In some ways PSAC is less important than it was since Congress is beginning to take an interest in the questioning and analysis of defence issues which was formerly done by PSAC or not at all. The panel report on 2,4,5-T suggests that PSAC, in trying to extend its sphere of authority to the civilian issues more usually covered by the National Academy of Sciences, has yet to acquire the tact and urge for completeness with which the academy seeks to hallmark its public reports.

DEFENCE SPENDING

FAS Flays Foster

by our Washington Correspondent

"MANY new occurrences inevitably come with spring," Senator Stuart Symington told the Senate in March this year, "some pleasant—warmer weather, Japanese cherry blossoms; some unpleasant—proposed higher dangers to this country because of new developments in Soviet weaponry . . . . In reasonably good humour I say to my colleagues and the taxpayer, 'Here we go again'". Within a few days of Senator Symington's observation, John S. Foster, the director of defence research and engineering in the Department of Defense, launched his springtime offensive against Congress on behalf of the $7,880 million the Pentagon is requesting for research and development in the coming financial year. The two prongs of his attack were that the Soviet Union is now spending the equivalent of $3,000 million a year more than the United States in these areas and that, as a result, "the Soviet Union could assume technological superiority in military research and development in the latter half of this decade".



John S. Foster, director of defence research and engineering. (From *The Washingtonian*.) (By John Lewis. ©1971 *Washingtonian Magazine*.)

The Senate Armed Services committee, before which Foster delivered this testimony, may well have been impressed not only by the bad news but also by Foster's magnificent forbearance in not demanding an extra $3,000 million for himself. Last week, in an unprecedented open hearing, the ad hoc subcommittee on research and development of the Senate Armed Services committee had the chance of hearing Foster's claims dissected and dismissed by the Federation of American Scientists. Pentagon statements about Soviet research, the federation said in a 50-page report entitled *Is there a Research Gap?*, have amounted to a "classical numbers game featuring selective disclosure, questionable assumptions, exaggeratedly precise estimates, misleading language and alarming non-sequitur conclusions".

The federation, a small but energetic lobbying group, devotes most of its energy to issues of arms control and disarmament. Its membership, composed almost entirely of scientists, includes several former members of the President's Science Advisory Committee. The analysis of the Defense Department's new claims about Soviet military research spending was put together by a federation committee which included Professor Marvin L. Gold-

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RESULTS OF THE PILOT STUDY ON THE ABSORPTION AND EXCRETION OF 2,4-D and 2,4,5-T BY HUMAN SUBJECTS

A report of the results of the indicated study is enclosed. Considering the quality of the subjects, as well as the investigators, the data were quite good. I feel that this study should be expanded to include 3 more subjects for each compound. Recommendations for future work are given at the end of the report.

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slk

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K-4568, PJ T23.14-23-23 (2,4,5-T)

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RESULTS OF A PILOT STUDY ON THE ABSORPTION AND EXCRETION OF 2,4-DICHLOROPHENOXY ACETIC ACID (2,4-D) AND 2,4,5-TRICHLORO-PHENOXY ACETIC ACID (2,4,5-T) FOLLOWING A SINGLE ORAL DOSE TO HUMAN SUBJECTS

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Toxicology

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Corporate Medical

April 27, 1971

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DOW 026822

OBJECTIVE

The objectives of the work reported herein were two-fold: (1) to determine the concentrations of 2,4-D and 2,4,5-T in blood as a function of time following oral administration, and (2) to determine the amount of these agents excreted in the urine during the first 48 hours following administration.

METHODS

Two human male volunteers weighing 76.8 and 81.0 kg ingested 5 mg/kg 2,4-D (Sample AGR 30653C) and 2,4,5-T (Sample AGR 86187), respectively. For ingestion, the samples, which were analytical standards prepared by The Dow Chemical Company, were mixed in milk. Blood samples for compound analysis, 10 ml, were collected immediately prior to ingestion and at 2, 7, 12, 24 and 48 hours following ingestion. Samples of the urine, collected 0 to 23, 23 to 24, 24 to 47 and 47 to 48 hours following administration, were used for compound analysis. Analyses for 2,4-D and 2,4,5-T (R. Papenfuss, AL28-109, 1971) were performed as described by Marquardt, 1970 (Dow Report ML-AM-70-95).

RESULTS AND DISCUSSION

The concentrations of 2,4-D and 2,4,5-T in the blood at various times following administration are given in Table 1. Before doing a kinetic analysis of the data, the concentration in plasma was calculated (see footnote to Table 1). This transformation was needed to derive a volume of distribution from the kinetic analysis.

In previous studies conducted in this laboratory, as well as in other laboratories (Courtney, 1970; Erne, 1966a), the disappearance of 2,4-D and 2,4,5-T from the plasma of rats, pigs, calves and chickens followed first order rate kinetics. Therefore, the kinetic model describing the concentration of these agents in plasma as a function of time was assumed to be that given by the equation

$$C_s = \frac{A_o k_f}{V_d(k_f - k_e)} \left(e^{-k_e t} - e^{-k_f t}\right)$$

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where
k_f = the apparent first order absorption constant (hr^{-1})
k_e = the apparent first order disappearance constant (hr^{-1})
V_d = the apparent volume of distribution (l/kg)
A_o = the dose administered (mg/kg)
t = time (hrs)
C_s = drug concentration (μgm/ml)

The values of k_f, k_e and V_d which provide the curves of best fit for the experimental data were determined using a computer program recently developed by G. E. Blau (NCM-10526, 1970). The concentrations of 2,4-D and 2,4,5-T found in plasma together with the curves of best fit are illustrated in Figures 1 and 2, respectively.

The rate constants (k_e) for the elimination of 2,4-D and 2,4,5-T were 0.035 ± 0.001 and 0.045 ± 0.004 hr^{-1}. These values correspond to a half-life ($t_{\frac{1}{2}}$) of 19.8 and 15.4 hours, respectively. In other species (rat, pig, calf, chicken) the $t_{\frac{1}{2}}$ ranges from 3 to 12 hours (Erne 1966a).

The volumes of distribution (V_d) for 2,4-D and 2,4,5-T were 0.114 ± 0.001 and 0.066 ± 0.002 l/kg. The plasma volume of man is 0.04 l/kg and the extracellular volume is 0.18 l/kg. Therefore, these data suggest that the distribution of these agents is extracellular. Since these agents are bound to plasma protein (Erne, 1966b), a volume of distribution less than 0.18 l/kg is expected. In previous studies using rats, the volume of distribution of 2,4-D was found to be 0.11 l/kg.

The excretion of 2,4-D and 2,4,5-T in the urine is depicted in Tables 2 and 3, respectively. Within 48 hrs, 94.4% of the dose of 2,4,5-T was excreted in the urine of the individual receiving this agent. Only 72.4% of the dose was excreted in the urine of the subject given 2,4-D. This difference is very likely an individual difference rather than a difference in the excretion of the compounds. In a recent study using rats, it was found that as much as 22% of the 2,4-D excreted may be excreted via the feces. Therefore, the frequency of defecation may influence the amount excreted in the urine as well as the rate of clearance from the body.

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In conclusion, the following suggestions are made for future studies.

1. Additional blood samples should be obtained at 32, 56 and 72 hours following the administration of the compounds.

2. The analyses for 2,4-D and 2,4,5-T should be conducted on plasma rather than on whole blood.

3. The plasma protein binding of 2,4-D and 2,4,5-T should be determined.

4. Urine should be collected from 48 to 72 and 72 to 96 hours following administration.

5. Feces should be collected from 0 to 24, 24 to 48 and 48 to 72 hours following administration.

REFERENCES

Courtney, K. D. 2,4,5-T in the rat: Excretion patter, serum levels, placental transport, and metabolism. Pesticide Symposia, Inter-American Conferences on Toxicology & Occupational Medicine, University of Miami School of Medicine, 1970.

Erne, K. 1966A. Distribution and elimination of chlorinated phenoxyacetic acid in animals. Acta vet Scand. 7:240-256.

Erne, K. 1966b. Studies on the animal metabolism of phenoxy-acetic herbicides. Acta vet Scand. 7:261-271.

Table 1

CONCENTRATION OF 2,4-D AND 2,4,5-T IN THE BLOOD OF MAN
AS A FUNCTION OF TIME FOLLOWING THE ADMINISTRATION OF 5 MG/KG

Time (hrs.) post-administration	2,4-D		2,4,5-T	
	µgm/ml blood	µgm/ml plasma[1]	µgm/ml blood	µgm/ml plasma[1]
0	0.0	0.0	0.0	0.0
2	21.0	35.3	25.0	42.0
7	20.0	33.6	33.0	55.5
12	18.0	30.3	30.0	50.5
24	15.0	25.2	19.0	32.0
48	2.1	3.5	3.9	6.5

[1] The ratio of the concentration of 2,4-D between plasma and red cells at equilibration is approximately 1:10 (Erne, 1966). Since the hematocrit of both subjects was 45%, the concentration of 2,4-D and 2,4,5-T in plasma was estimated using the following equation:

$$0.55y + 0.45\ (1/10y) = C$$

where y is the concentration in plasma and C is the concentration in blood.

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Table 2

URINARY EXCRETION OF 2,4-D BY A MAN GIVEN 384 MG, 5 MG/KG

Duration of collection, Hr post-administration	Volume of urine voided ml.	Volume of urine following dilution ml.	2,4-D concentration µgm/ml	2,4-D Excreted		
				Total, mg	%	Cumulative %
0 to 23	1320	1500[1]	79.0	118.5	30.9	30.9
23 to 24	56	56	98.0	5.5	1.4	32.3
24 to 47	1400	1500[1]	99.0	148.5	38.7	71.0
47 to 48	180	180	29.0	5.2	1.4	72.4

[1] Sufficient water was added to the undiluted urine sample to give the volume indicated.

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Table 3

URINARY EXCRETION OF 2,4,5-T BY A MAN GIVEN 405 MG, 5 MG/KG

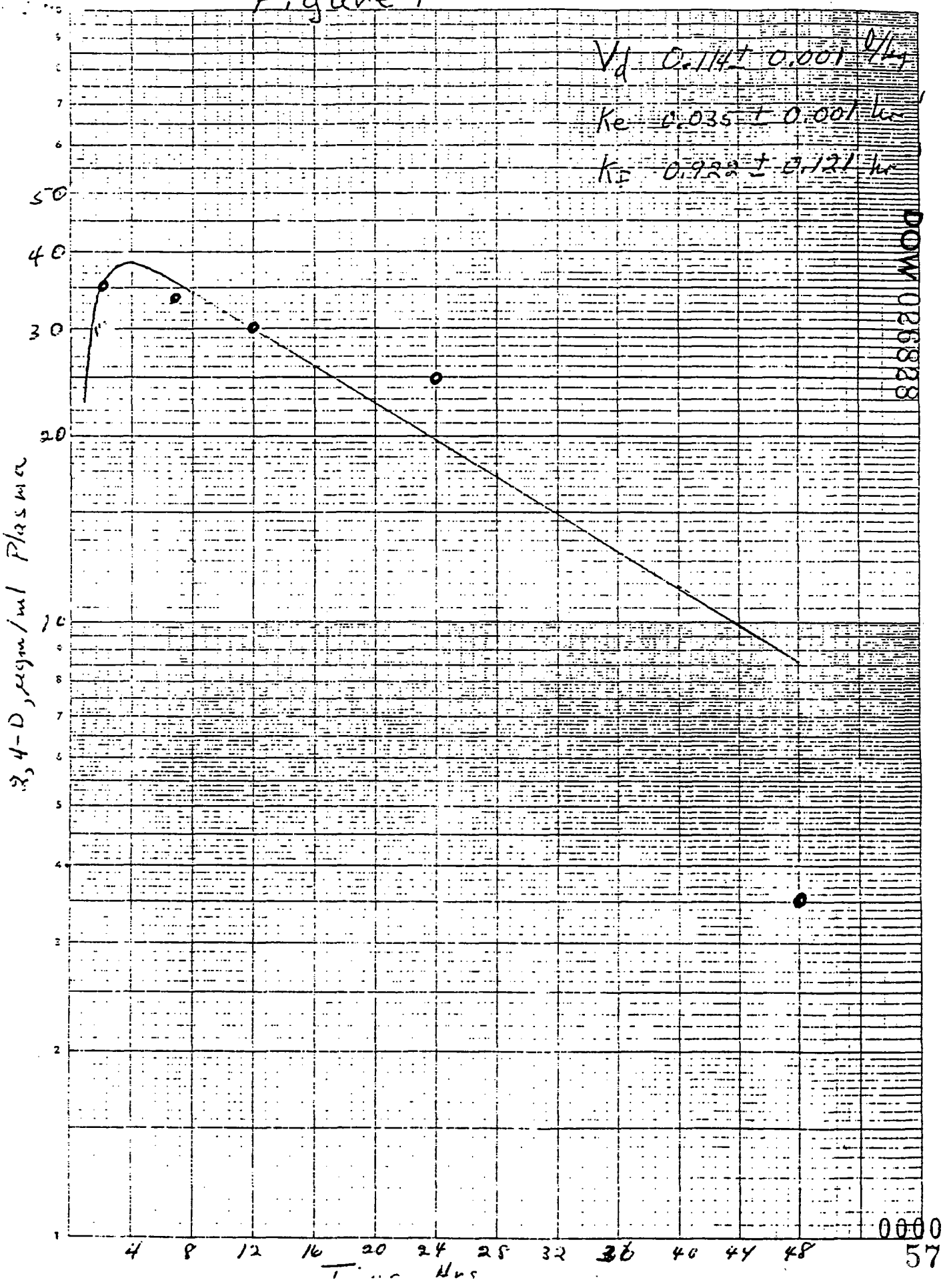
Duration of collection, Hr post-administration	Volume of urine voided ml.	Volume of urine following dilution ml.	2,4,5-T concentration µgm/ml	2,4,5-T Excreted		
				Total, mg	%	Cumulative %
0 to 23	1680	2000[1]	105.0	210.0	51.8	51.8
23 to 24	103	103	97.0	10.0	2.5	54.3
24 to 47	2340	2340	68.0	159.1	39.2	93.5
47 to 48	204	204	17.0	3.5	0.9	94.4

[1] Sufficient water was added to the undiluted urine sample to give the volume indicated.

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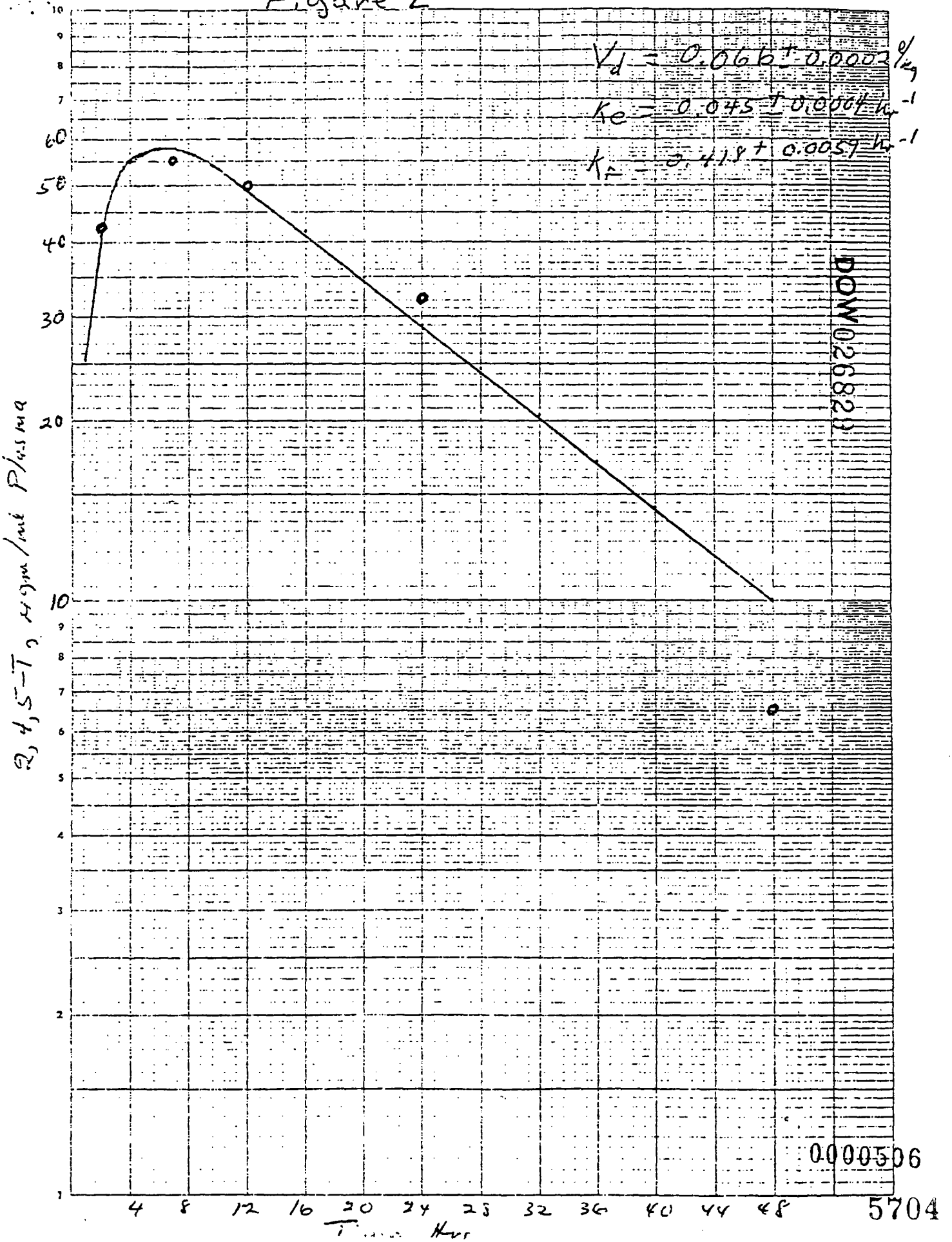
Figure 1



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Figure 2



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T36.25-66681-14

August 30, 1971

A PROBE STUDY OF THE ACUTE ORAL TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN THE CANINE

By: G. L. Sparschu, J. E. Bourne, P. J. Gehring and J. M. Norris

DOW 749678

OBJECTIVES

This study was conducted to define a range of toxicity from a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCBD) in the canine. In addition, clinical and pathological evaluations were conducted in an effort to further define the toxic properties of TCBD. This study is intended to be a probe study and the data must be interpreted in this context.

EXPERIMENTAL METHODS

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCBD), reference number 340-2-54B, was administered to unconditioned pound dogs. The study was conducted in two parts; first, male dogs, 2/treatment level, were given 3 mg/kg or 300 µg/kg TCBD and 2 months later female dogs, 2/treatment level, were given 100 or 30 µg/kg TCBD.

The TCBD was suspended in an 8:2 corn oil-acetone solution and administered as a single oral dose in gelatin capsules. The dogs ranged in weight from 8.0 to 17.5 kg and were given from 8-17 ml of the TCBD corn oil-acetone mixture.

In the male dogs, evaluations of blood urea nitrogen (BUN), serum alkaline phosphatase (AP) and serum glutamic pyruvic

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transaminase (SGPT) were made on test days 1, 8, and 15.

Hematology studies consisting of packed cell volume, hemoglobin, total erythrocyte count and total leukocyte count and differential were conducted on test day 18 for the two male dogs given 300 µg/kg of TCBD and on test day 64 for all the female dogs.

Body weights were taken on all dogs prior to dosing and weekly thereafter. Complete gross and histopathology were conducted on all dogs.

RESULTS AND DISCUSSION - MALE DOGS

The clinical signs for all four dogs, regardless of dose, were essentially the same. The clinical signs consisted of anorexia, dehydration, depression, emaciation, and gastrointestinal hemorrhage. The two dogs given 3 mg/kg of TCBD died after 9 or 15 days on test. Those dogs given 300 µg/kg of TCBD were killed after 18 or 22 days on test due to their extreme cachexic and moribund condition.

The clinical chemistry results are given in Table 1. All dogs showed slight to moderate elevations in SGPT and dog 70-3171 had a terminal elevation of BUN.

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The hematology results are given in Table 2. Dog 70-3170 had a decreased PCV and both male dogs had a leukocytosis.

Table 3 contains the gross and histopathology results. The digestive tract appears to be particularily sensitive to the affects of TCBD resulting in focal areas of inflammation, necrosis, hemorrhage and ulceration. The major organ affected was the liver; focal areas of necrosis, usually central lobular with only a minimal number of hepatocytes involved. Another treatment related observation is the vasculitis that is most prominent in the omental and mesenteric vessels. An adjacent steatitis accompanied the vasculitis. The profound anorexia that the TCBD produced resulting in extreme emaciation and dehydration no doubt was a major contributing factor in the death or moribund condition of the male dogs ~~===~~ receiving 30 mg or 300 µg/kg TCBD.

RESULTS AND DISCUSSION - FEMALE DOGS

The first several days after treatment the dogs appeared clinically normal. By eleven days they were dehydrated, anorexic, depressed, and had intestinal hemorrhage. Dog 71-424 (30 µg/kg) appeared near death and had a generalized partial alopecia. Dog 71-425 (100 µg/kg) had minimal shedding of hair and both dogs given 100 µg/kg TCBD had unsteady

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gaits. At fourteen days the dogs still had evidence of intestinal hemorrhage; the condition of dog 71-424 (30 µg/kg) was unchanged. Dog 71-425 (100 µg/kg) was not showing extensive alopecia and dog 71-423 (30 µg/kg) was starting to shed hair. At 18 days and thereafter, there was no evidence of intestinal hemorrhage and all four dogs appeared more alert suggesting partial recovery.

The dogs treated with 100 µg/kg TCBD continued to lose body weight the first 30 days after which they began to gain weight; by 64 days they were approaching their pre-test body weight (Figure 1). The dogs given 30 µg/kg TCBD also lost body weight, but after 15 days they began to gain back their weight.

The hematology results, Table 2, show a decreased PCV, total erythrocyte count, and hemoglobin with a leukocytosis. The decrease in erythrocitic values is probably a reflection of the intestinal hemorrhage clinically observed. Terminal hematoxylin and eosin stained sections of bone marrow were unremarkable; there was no evidence of bone marrow depression.

The pathology results are given in Table 4. Various degrees of alopecia were seen in all dogs, with the 100 µg/kg treated

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dogs showing the most hair loss. Microscopically the skin lesions consisted of a folliculitis with epidermal inflammatory cells and focal ulceration. There was no marked hyperkeratosis or keratotic cyst formation to relate the skin lesions to chloracne.

Dog 71-426 (100 μg/kg) had scattered inflammatory cells in the hepatic sinusoids and dog 71-425 (100 μg/kg) had a mild chronic colitis.

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SUMMARY

Two unconditioned male pound dogs/group were treated with 3 mg/kg or 300 µg/kg of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCBD). The TCBD was administered as a single oral dose suspended in an 8:2 corn oil-acetone solution. All male dogs became anorexic, depressed, emaciated and had gastrointestinal hemorrhage. The dogs receiving 3 mg/kg died on test days 9 and 15 and those receiving 300 µg/kg were killed on days 18 and 22 because of their moribund condition. Lesions noted in the digestive tract included ulceration, inflammation, necrosis and hemorrhage. All dogs had minimal to moderate amounts of hepatic necrosis. There was a vasculitis present, especially in the omental and mesenteric vessels, with an adjacent steatitis. The profound anorexia and dehydration were important contributory factors as to the cause of death or moribund condition of the male dogs treated with 3 mg/kg or 300 µg/kg of TCBD.

Two unconditioned female pound dogs/group were treated with 100 µg/kg or 30 µg/kg of TCBD. Several days after treatment they became anorexic, dehydrated, depressed, lost weight and had intestinal hemorrhage. Approximately eighteen days after treatment the dogs became more alert, began eating and

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intestinal hemorrhage stopped suggesting the beginning of recovery. The dogs receiving 100 µg/kg started to show weight gains 30 days after treatment and those receiving 30 µg/kg showed weight gains after 15 days. All female dogs had various degrees of alopecia with the 100 µg/kg level being the most severe. Histopathologically, the skin lesions consisted of inflammatory cells in the epidermis and a folliculitis. The female dogs were killed 64 days after treatment and at this time one dog at the 100 µg/kg dose had a mild chronic colitis and the other dog at this dose had scattered inflammatory cells in the hepatic sinusoids.

It is concluded that single oral doses of 300 µg/kg and above of 2,3,7,8-tetrachlorodibenzo-p-dioxin are lethal in the dog. Single oral doses of 100 µg/kg and 30 µg/kg of 2,3,7,8-tetrachlorodibenzo-p-dioxin are severely toxic to the dog, but recovery does occur.

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TABLE 1

STUDY: Single Dose Oral Probe
TEST MATERIAL: 2,3,7,8-Tetrachlorodibenzo-p-dioxin
TEST ANIMAL: Unconditioned Pound Dog - Male

CLINICAL CHEMISTRY, BODY WEIGHT AND MORTALITY DATA

Dose and Animal Number	Day of Test....	BUN (mg%)			AP (K.A. Units)			SGPT (Karmen Units/ml)			Body Weight Loss (kg)			Test Day of Death
		1	8	15	1	8	15	1	8	15	8	18	22	
3 mg/kg														
70-3167		11	25	--	10.9	32	--	56	92	---	2.1	---	---	9
70-3168		18	18	--	10.9	6	--	68.7	53	---	0.6	---	---	15
300 µg/kg														
70-3170		14	21	15	8	7	5	58	32	60	1.3	2.8	3.4	22*
70-3171		15	17	90	11	15	20	53	56	100	1.2	4.2	---	18*

*Killed because of moribund condition.

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TABLE 2

STUDY: Single Dose Oral Probe
TEST MATERIAL: 2,3,7,8-Tetrachlorodibenzo-p-dioxin
TEST ANIMAL: Unconditioned Pound Dog

HEMATOLOGY SUMMARY

Dose and Animal Number	Sex	Test Day	PCV %	RBC x 10^6	Hemoglobin g/100 cc	WBC x 10^3	Differential Count (%)					
							Neut		Lymph	Mono	Eosin	Baso
							Seg.	B/J				
300 µg/kg												
70-3170	M	18	39	5.9	14.2	27.1	70	10	16	4	0	0
70-3171	M	18	54	7.3	19.8	31.2	79	5	9	7	0	0
100 µg/kg												
71-425	F	64	32	4.9	11.6	22.1	33	0	15	10	42	0
71-426	F	64	33	5.3	11.9	24.2	51	3	21	11	14	0
30 µg/kg												
71-423	F	64	35	5.9	12.6	24.8	63	1	22	7	7	0
71-424	F	64	30	5.1	10.8	20.9	53	3	31	6	7	0

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TABLE 3

STUDY: Single Dose Oral Probe
TEST MATERIAL: 2,3,7,8-Tetrachlorodibenzo-p-dioxin
TEST ANIMAL: Unconditioned Pound Dog - Male

GROSS AND HISTOPATHOLOGY SUMMARY

imal mber	Dose	Gross Pathology	Histopathology
-3167	3 mg/kg	Died, cachexic; buccal ulcers; liver--congested with lobular accentuation; kidneys--swollen; omentum--white lobulated mass; adrenals--enlarged; gastric ulcers; GI tract empty and congested.	Thrombus in renal arcuate artery; splenic lymphoid depletion and focal hemorrhage; diffuse pulmonary congestion; vasculitis of vessel in coronary groove; liver--central lobular congestion with diffuse focal hepatocyte necrosis and leukocyte infiltration; enterocolitis; omental steatitis; a nonspecific vasculitis mainly involving the arteries in the omentum and mesentery; focal necrosis and leukocyte infiltration of adrenal gland adjacent to necrotizing vasculitis; mesenteric lymph node--lymphoid depletion and perilymphadenitis adjacent to the vasculitis of the surrounding adipose tissue.
3168	3 mg/kg	Died, cachexic, Dirofilaria, liver--pale and granular; perirenal edema and renal swelling; mesenteric lymph node hemorrhagic; omentum--lobulation of adipose tissue; GI tract--edema, congestion and focal hemorrhage.	Chronic prostatitis; acute inflammation of adrenal with periadrenal hemorrhage; chronic nephritis; lung--vasculitis with Dirofilaria; mesenteric lymph node--lymphoid depletion, hemorrhage, edema and vascular necrosis; spleen--lymphoid depletion; liver--central lobular necrosis and fatty metamorphosis; omentum--steatitis; acute enteritis with hemorrhage and necrosis.

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CONTINUATION OF TABLE 3

imal mber	Dose	Gross Pathology	Histopathology
-3170	300 µg/kg	Killed, cachexic; moribund; buccal ulcers; intestinal hemorrhage; liver--mottled.	Acute prostatitis; liver--central lobular necrosis; acute myosititis of adductor group; enteritis with ulceration and necrosis; omentum--steatitis; chronic colitis.
-3171	300 µg/kg	Killed, cachexic; moribund; Dirofilaria; urinary bladder--serosal hemorrhages; gall bladder--edematous and distended; omentum--lobulation of adipose tissue; GI tract--edematous with focal serosal and mucosal hemorrhages.	Spleen--lymphoid depletion; lung--vasculitis with Dirofilaria; acute esophagitis, focal myocarditis, renal abscessations, liver--fatty metamorphosis; omentum--steatitis and vasculitis; serosal surface of abdominal viscera--chronic hemorrhage with fibroblast proliferation.

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TABLE 4

STUDY: Single Dose Oral Probe
TEST MATERIAL: 2,3,7,8-Tetrachlorodibenzo-p-dioxin
TEST ANIMAL: Unconditioned Pound Dog - Female

GROSS AND HISTOPATHOLOGY SUMMARY

imal mber	Dose	Gross Pathology	Histopathology
-425	100 µg/kg	Skin--symmetrical bilateral alopecia of abdomen, sides, and legs with erythematous papulen and focal scab formation in areas of alopecia.	Chronic colitis-minimal; chronic lymphadenitis of mesenteric lymph node; skin--focal ulceration with acute and chronic folliculitis.
-426	100 µg/kg	Skin--symmetrical bilateral alopecia of abdomen, sides and legs with erythematous papulen and focal scab formation in areas of alopecia; focal ulcerations on hard and soft palates; renal capsule thickened.	Focal ulceration, necrosis and hemorrhage in palatine tissues; chronic interstitial nephritis with focal parasitic granuloma; liver--acute and chronic inflammatory cells in sinusoids; skin--focal ulceration with acute and chronic folliculitis.
-423	30 µg/kg	Skin--ventral alopecia with minimal pustular scab formation; Dirofilaria, intestinal tapeworms.	Focal chronic interstitial nephritis; focal dermatitis with folliculitis-minimal.
-424	30 µg/kg	Skin--minimal alopecia of front legs with slight erythema and papule formation.	Dermatitis with focal ulceration-minimal.

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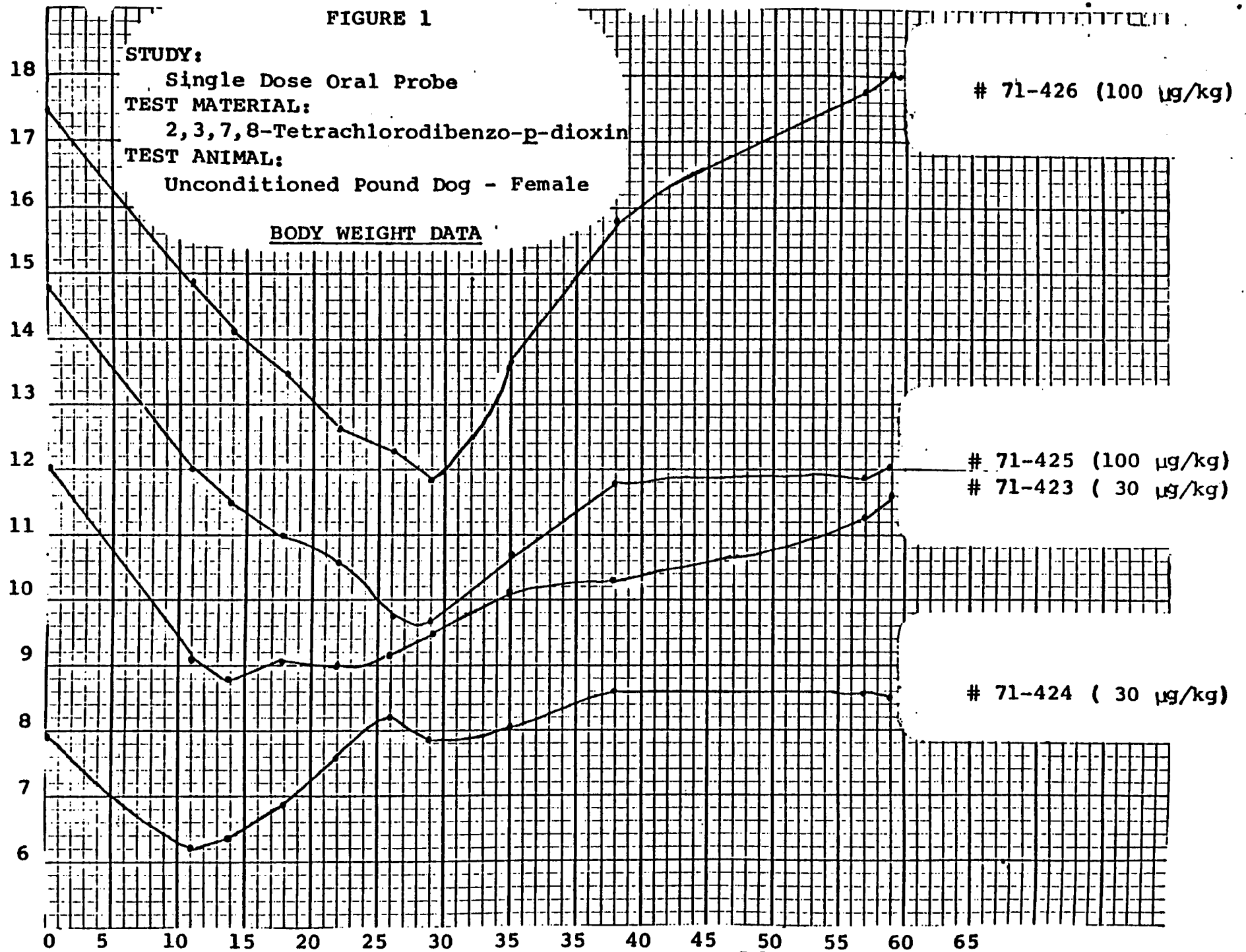
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FIGURE 1

STUDY:
Single Dose Oral Probe
TEST MATERIAL:
2,3,7,8-Tetrachlorodibenzo-p-dioxin
TEST ANIMAL:
Unconditioned Pound Dog - Female

BODY WEIGHT DATA



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Reprinted from *BioScien*

The Public Health Implications of Widespread Use of the Phenoxy Herbicides and Picloram

Julius E. Johnson

This paper is concerned primarily with 2,4-D, 2,4,5-T, silvex, and picloram—the phenoxies because of widespread use; picloram, 2,4,5-T, and 2,4-D more specifically because of their use as military defoliants. The purpose of this discussion is to explore possible public health consequences of widespread use. This presentation will be confined primarily to information as it may translate to the health and safety of man. The data presented will emphasize exposure, toxicity, and chemistry.

First, I will comment on exposure to food, water, and air. There is very little exposure of man to the phenoxy herbicides through food. The principal uses of these herbicides in growing food crops were originally registered on a no-residue basis. A change in policy now requires these registrations be supported by a negligible residue tolerance. An industry task force has formally requested FDA to establish these tolerances. (The request for 2,4,5-T has been temporarily withdrawn without prejudice to future filing.)

TABLE 1.- Tolerances proposed to FDA

	2,4-D	2,4,5-T	MCPA	Silvex	Picloram
Fruits	X	X		X	
Berries	X	X			
Grains	X	X	X	X	
Rice	X*	X	X	X	
Flax Seed	X*		X		
	X				
Potatoes	X	X		X	
Sugarcane	X		X		
Hay					X*
Forage Grasses					X*
Meat					X
Milk					

(All negligible residue tolerances except those marked*)

Table 1 shows a condensed version of tolerances proposed for 2,4-D, 2,4,5-T, MCPA, silvex, and picloram. Those marked with an asterisk are for tolerances at a permissible level for 2,4-D in rice and flax seed and for picloram in forage grasses and meat. The remainder are negligible residue tolerances.

Table 2 demonstrates the infrequency of residues of phenoxy herbicides in foods as confirmed by the FDA market basket survey of pesticides in composites of 12 categories of foodstuffs. Of the 1548 samples analyzed, only 36 positive identifications were made. All but one was less than 0.2 ppm. 2,4,5-T was found in three of the surveyed samples (Duggan et al., 1967; Martin and Duggan, 1968; Corneliussen, 1969).

Air and water are potential sources of exposure of man to these herbicides. Manigold and Schulze (1969) have reported on pesticide residues in selected streams in the West such as the Missouri, Yellowstone, Colorado, Arkansas, Rio Grande, Snake, and others. Phenoxy herbicides were detected as shown in Table 3. Of the 320 samples taken, fractional parts per billion concentrations were reported in 78 samples. The measurement of picloram residues in water has been reported following *direct spraying of a static* pond in Texas (Hoffman et al., 1969) and a *watershed* experiment was conducted in North Carolina using 2,4-D, 2,4,5-T, and picloram (Sheets and Lutz, 1969). The results of these studies are summarized in Table 4. It is noted that picloram persisted longer than 2,4-D or 2,4,5-T. Air samples collected in spring and summer from two wheat-growing areas in the State of Washington were analyzed for phenoxy herbicides by Bamesberger and Adams (1966). The results are summarized in Figure 1. Phenoxy herbicides are widely used for early postemergence control of weeds in these crops. There were detectable levels of 2,4-D and 2,4,5-T in the air up to 0.06 $\mu g/m^3$. Man will inhale about 30 m^3 of air per day. At a level of 0.06 $\mu g/m^3$, this would amount to an exposure of

TABLE 2. FDA market basket survey pesticide residues 1965-68

	Number of Samples with Residues of 1548 Samples Analyzed			
	PPM			Total
	0.001 – <0.1	0.1 – <0.2	0.4	
2,4-D	23	4	0	27
MCPA	2	1	1	4
2,4,5-T	2	1	0	3
Silvex	2	0	0	2

Pesticide Monitoring Journal, 1967, 1968, 1969

Duggan, R. E., H. C. Barry, and L. Y. Johnson, 1967. *Pesti*
Martin, R. J., and R. E. Duggan, 1968. *Pes*
Corneliussen, P. E. 1969. *P*

The author is Vice-President and Director of Research, Dow Chemical Company.

Presented at the Symposium on Possible Public Health Implications of Widespread Use of Herbicides (Chairman: Dr. Arthur Galston) American Institute of Biological Sciences, Bloomington, Indiana, 26 August 1970.

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CHEMICAL BIOLOGY RESEARCH | DOW CHEMICAL U.S.A. | FILE NB T36.25-66681-22

SUBMITTED BY	CHARGE	DATE	K NUMBER
	01269-000-026	10/25/71	K-66681

EYE IRRITATION STUDY CONDUCTED ON:

2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

REPORTED BY: J. M. Norris CHECKED BY: P. J. Gehring

INFORMATIVE SUMMARY WITH CONCLUSIONS BASED ON THE SAMPLE RECEIVED. ADDITIONAL INFORMATION INCLUDING THE EFFECTS REPEATED EXPOSURE MAY BE REQUIRED AS SPECIFIC USES AND FORMULATIONS ARE DEVELOPED OR IF PROCESS CHANGES OCCUR

A sample of 2,3,7,8-tetrachlorodibenzo-p-dioxin, identified by #340-2-54B, was submitted to Chemical Biology Research for evaluation of eye irritation properties.

Twenty-four hours prior to use, the eyes of an albino rabbit were examined and established as being without defects or irritation. The test procedure involved instillation of 2 mg of the test compound into the conjunctival sac of the left eye. The right eye was used as an untreated control. The treated eye was examined for conjunctival inflammation, iritis and corneal injury immediately after instillation, at 1, 24 and 48 hours and 13, 22 and 27 days post-instillation. The behavior of the animal was observed for indication of pain or discomfort at the time of instillation.

The compound caused discomfort on instillation and very slight irritation of the conjunctival membranes which persisted for 24 hours. Delayed conjunctival chemosis causing the eye lids to be more than half closed was observed on day 13 and by day 22 the condition was severe. On day 27 the chemosis had subsided, however, the eye lid rim was thickened and encrusted.

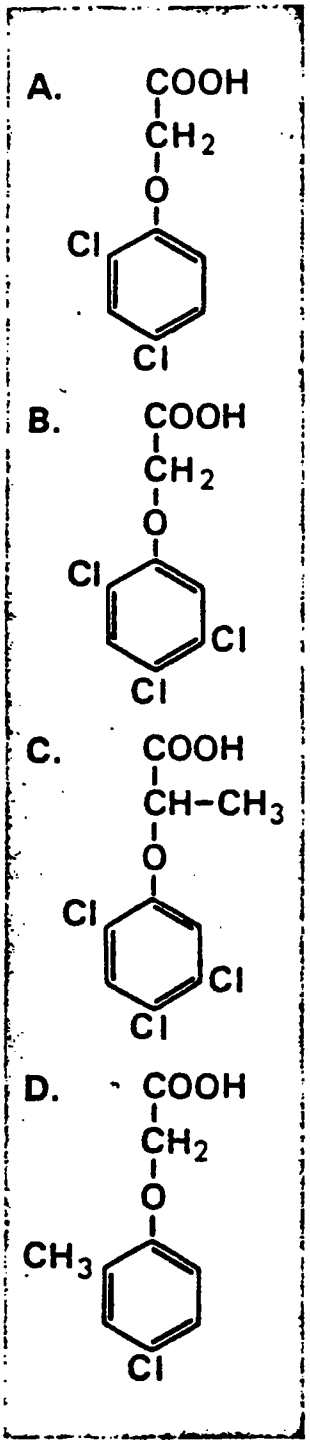
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A. 2,4-dichlorophenoxyacetic acid
B. 2,4,5-trichlorophenoxyacetic acid
C. 2-(2,4,5-trichlorophenoxy)-propionic acid
D. 2-methyl-4-chlorophenoxyacetic acid

The status of 2,4-D, 2,4,5-T, Silvex and MCPA Herbicides

By C. S. Williams
R & D Herbicide Tech. Specialist,
The Dow Chemical Company,
Midland, Michigan.

BACKGROUND

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was developed in the mid-forties and was the forerunner of a group of phenoxy herbicides that have been instrumental in the control of broadleaved weeds in food crops and undesirable brush species on industrial rights-of-way. In addition these products have contributed immeasurably to beef production by controlling weed and/or brush on pasture and rangeland, resulting in increased grass production and corresponding increase in carrying capacity for livestock.

Besides 2,4-D, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-(2,4,5-trichlorophenoxy) propionic acid (silvex or 2,4,5-TP), and 2-methyl-4-chlorophenoxy acetic acid (MCPA) are major phenoxy products of similar chemical structure, as shown below, but with unique characteristics of their own with respect to species controlled and crop selectivity.

CHRONOLOGY OF EVENTS

Over the years there has been considerable improvement in phenoxy herbicides and their use. Development of new formulations, performance information, crop safety, timing of application, spray equipment, toxicology, use hazards, and environmental implications have contributed to both better product and specific directions for use.

These herbicides are not protected by patents, so seven commercial companies were manufacturing one or more of the four phenoxies (2,4-D, 2,4,5-T, silvex, MCPA) at the time when the USDA announcement to abolish the no residue status was issued on April 13, 1966. Basic manufacturers included: Diamond, Dow, Hercules, Monsanto, Rhodia (then Chipman), Thompson, and Thompson-Hayward. These com: joined to form the Industry Task Fo Phenoxy Herbicide Tolerances (IT The chronology of major events assc with this has been as follows:

April 13, 1966
USDA announcement to abolish n due status—industry must comply taining tolerances for residues in all t food and feed products and byprodu December 31, 1970.

August 23, 1966
Industry Task Force on Phenoxy Her Tolerances was formed to handle 2,4,5-T, MCPA and silvex.

December, 1967
Submitted petitions to FDA for tole of 2,4-D, 2,4,5-T, silvex and MCPA ing all food crop uses listed at that t the USDA Summary of Registered A tural Chemical Uses. Extension of re tion was requested for uses of these cides in pasture and rangeland.

April, 1968
Industry Task Force advised of i quacies, in the petitions plus require for information on all metabolites herbicides that might occur as resid food.

September, 1968
Review of literature on metabolism mitted to resolve metabolite question.
During 1968 programs were estab to determine on which crops add work would be undertaken and what sp projects would be done by each cor in the Task Force.

October 29, 1968
Industry Task Force requested exte for continued use of 2,4-D, 2,4,5-T, s and MCPA on pastures and rangelan tension was granted until January 1,

January 31, 1969
Use of 2,4-D, 2,4,5-T and silvex on ac sites extended to January 1, 1970.
Crop samples for residue analyses collected during summer and fall of
Protocols for milk and meat studies established during 1969. It was decide analyses of animal tissues would inclu phenol corresponding to each phenoxy pound. Dow handled dairy cattle feedir milk analysis for all four phenoxie began feeding February, 1970.
USDA was scheduled to handle feed phenoxies to beef cattle and sheep c winter, 1969-1970.

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TABLE 8
The Effect of Resistant Variety and Crop Rotation on Wilt Control and Performance 1969 (Pitt, Wake and Vance Counties)

Variety	No Rotation Wilt[1] Index	No Rotation Value $/Acre	Rotation Wilt[1] Index	Rotation Value $/Acre	$ Increase By Rotation
Coker 254	17	975	16	1243	268
N. C. 2512	74	647	43	1005	358
Speight G-36	33	734	28	1091	357
Coker 319	79	618	61	884	194

[1] Wilt Index indicates extent of root damage. 0 = no damage, 100 = maximum disease development

TABLE 9
The Effect of Resistant Variety and Chemical Soil Treatment with TELONE C on Wilt Control and Performance (Pitt, Wake and Vance Counties)

Variety	No Fumigation Wilt[1] Index	No Fumigation Value $/Acre	Fumigation Wilt[1] Index	Fumigation Value $/Acre	$ Increase By Fumigation
Coker 254	17	975	13	1227	252
N. C. 2512	74	647	62	885	238
Speight G-36	33	734	26	1037	303
Coker 319	79	618	75	744	134

[1] Wilt Index indicates extent of root damage. 0 = no damage, 100 = maximum disease development.

TABLE 10
The Effect of Resistant Variety, Crop Rotation and Chemical Soil Treatment with TELONE C on Wilt Control and Performance (Pitt, Wake and Vance Counties)

Variety	Wilt Index[1] No Rotation	Wilt Index[1] Rotation & Fumigation	Value $/Acre No Rotation	Value $/Acre Rotation & Fumigation	Value $/Acre Increase
Coker 254	17	18	975	1283	308
N. C. 2512	74	47	647	1049	402
Speight G-36	33	18	734	1212	478
Coker 319	79	61	618	970	352

[1] Wilt Index indicates extent of root damage. 0 = no damage, 100 = maximum disease development.

TABLE 11
The Effect of Crop Rotation & Chemical Soil Treatment with TELONE C on Control of Root Knot and Black Shank in Two Resistant Varieties (Columbus, Edgecombe and Jones Counties)

1968 Crops	Fumigation	Variety – Speight G-36 Root Knot Index[1]	Variety – Speight G-36 Black Shank Index[1]	Variety – Speight G-36 $ Value	Variety – Coker 254 Root Knot Index[1]	Variety – Coker 254 Black Shank Index[1]	Variety – Coker 254 $ Value
Small Grain Fescue	None	46	25	1137	8	8	1244
Corn	None	71	32	1062	8	5	1296
Cotton	None	41	18	1135	6	9	1251
Soybeans	None	41	18	1140	1	6	1259
Peanuts	None	31	14	1159	9	6	1223
Sweet Potatoes	None	61	34	11-2	8	5	1349
Average of all Rotation	None	48	24	1122	6	7	1278
Tobacco	None	61	45	931	3	7	1261
Tobacco	Telone C	48	24	1257	2	2	1389

[1] Root Knot and Black Shank Index indicates extent of root damage. 0 = no damage, 100 = maximum disease development

alone or continuous tobacco, plus TELONE C. These results suggest that a complete control program involving several practices is needed where resistant varieties (regardless of level) that are susceptible to the southern root-knot nematode are used. They also suggest that the use of a variety with high resistance to wilt and root knot might permit the omission of crop rotation or the use of a multi-purpose chemical soil treatment from the control program. Results are presented in Table 10.

A second series of integrated disease control tests was established in 1968 at three locations. These tests were located in Columbus, Edgecombe and Jones counties in fields where root-knot nematodes and black shank were present at low to moderate levels. Sixteen treatments involving seven two-year cropping systems were compared with continuous tobacco culture. Alternate crops included in the different cropping systems were corn, cotton, small grain fescue, soybeans, sweet potatoes and peanuts. Two disease resistant varieties were used within all cropping systems including Coker 254 (high resistance to black shank, bacterial wilt and root knot) and Speight G-36 (high resistance to black shank and bacterial wilt but susceptible to root knot). Two soil treatments (one using TELONE C soil fumigant and one using no soil treatment) were used in plots where continuous tobacco culture was practiced. No chemical soil treatment was used in rotated plots. Results from these tests provided a comparison of several alternate crops and TELONE C in reducing root knot and black shank and their effects on performance.

Disease development was low in the Coker 254 variety at all locations. Rotation with such crops as corn, cotton, peanuts, small grain fescue, soybeans and sweet potatoes failed to provide additional control or increase in performance of this variety. (Table 11).

The Speight G-36 variety was damaged severely by both root knot and black shank in most tests where tobacco was planted continuously. Black shank root damage was reduced by all cropping systems and root knot by all with exception of corn and sweet potatoes as compared with continuous tobacco culture. (Table 11).

The use of TELONE C in plots where tobacco was planted continuously gave equal disease control and a slightly higher value increase as compared with most cropping systems in both the Coker 254 and Speight G-36 varieties. (Table 11).

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October 29, 1969
The Office of Science and Technology issued statement on teratogenic hazard of 2,4,5-T based on work by Bionetics Research Laboratory.

December 22, 1969
Phenoxy registration for rangeland use extended until January 1, 1971, except for 2,4,5-T. Dow undertook feeding of 2,4,5-T to beef animals.

December 31, 1969
Petition for tolerances of 2,4,5-T in food crops was withdrawn by Industry Task Force since tolerances could not be established by January 1, 1970, the deadline set by the Office of Science and Technology.

January 19, 1970
Registration of 2,4-D, silvex and MCPA extended by USDA for use on food crops until January 1, 1971.

March 4, 1970
Registration of 2,4,5-T was also extended until January 1, 1971 for use on apples, blueberries, grains, pastures, rangeland, rice and sugarcane.

April 15, 1970
Results of additional work on teratogenic properties of 2,4,5-T prompted suspension by USDA of 2,4,5-T for aquatic and home uses.

May 1, 1970
Cancellation by USDA of 2,4,5-T for use on food crops. Uses on pasture, forests and industrial areas not affected.

May 28, 1970
Dow appealed the cancellation of 2,4,5-T for use on rice Hercules and Amchem also appealed cancellation for rice usage. Each company could appeal only crops listed on their labels for 2,4,5-T products. The appeal is to be reviewed by an Advisory Committee appointed by the National Academy of Sciences.

June 16, 1970
USDA began the beef feeding studies scheduled to have been done the previous winter.

November 24, 1970
Crop residue work completed. Milk analysis completed. Meat analyses underway. Completed data will be submitted as amendment to petitions prior to December 31, 1970.

To date, no official notice has been received concerning appointment of National Academy of Science Advisory Committee to review the appeal on the cancellation of rice.*

REGISTRATION STATUS

Tolerances have been established for 2,4-D in applies, barley, grapefruit, lemons, oats, oranges, pears, rye and wheat. There is also a tolerance for the sodium salt of 2,4-D in asparagus.

Data originally submitted with the petitions in December, 1967 is expected to be sufficient for 2,4-D in blueberries, cranberries, grapes and raspberries; for silvex in apples, pears and prunes; and for MCPA in peas. Data on residues in grass includes that from previous work and from 1970 residue samples additionally analyzed by Dow for support with respect to pasture and rangeland usage.

The Industry Task Force supported work for determining residues as shown in Table 1.

For virtually all of this work, new analytical methods had to be developed that would permit analyses down to 0.1 ppm phenoxy acid in crops and to 0.05 ppm acid or corresponding phenol in the animal tissues and milk.

For present registered uses residue analyses indicate < 0.2 ppm for all phenoxy acids in all crops at time of harvest.

The phenoxies were fed at levels of 30, 100 and 300 ppm for two weeks and 1000 ppm for three weeks in the total diet of dairy cows. Milk was collected and analyzed for residues. Grazing restrictions compatible with levels of phenoxies in milk as related to levels in forage remain to be determined. There was no evidence of accumulation of phenoxies in the cream.

At time of this writing, December 1, 1970, analyses are being run on samples of muscle, kidney, liver and fat of beef animals. Data are expected to be available prior to December 31, 1970.

Amendments to the petitions for tolerances for 2,4-D, silvex and MCPA will be submitted to the appropriate agency in charge, including uses in pasture and rangeland. The 2,4,5-T petition will be reactivated —deadline for this compliance will be accomplished prior to December 31, 1970.

TOXICOLOGY

Negligible residue tolerances can be obtained based on information from 90 day toxicology studies in two species of mammals. However, tolerances at higher (permissible) residue levels require two-year feeding studies on rats and dogs, plus fertility and reproduction studies on rats. At the time the phenoxy herbicides were

* See update at end of text (1).

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TABLE 1. Crops and tissues sampled for residue analysis (+) under the ITFPHT program.

	2,4-D	2,4,5-T	Silvex	MCPA
Corn	+	+	–	–
Rice	+	+	+	+
Flax	–	–	–	+
Small Grains (Barley, Oats, Rye, Wheat)	Tolerances Granted	–	–	+
Sorghum	+	–	–	–
Sugarcane	+	+	+	–
Milk	+	+	+	+
Meat	+	+	+	+

developed these long term feeding studies were not necessary, since these compounds were registered on a "no-residue" basis.

FDA has conducted two-year feeding studies on 2,4-D including reproduction and fertility. Dow has conducted two-year feeding studies on silvex but not reproduction or fertility studies.

Ninety-day feeding studies have been run on rats and dogs for 2,4-D, 2,4,5-T, silvex and MCPA. The no-ill effect levels are shown in Table 2.

Based on single oral doses in rats, 2,4-D, 2,4,5-T, silvex and MCPA are classed as "slightly toxic" with LD_{50} values ranging from three hundred to seven hundred mg/kg body weight.

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

The word teratology has recently become much more familiar. It was tied to 2,4,5-T when studies by Bionetics Research Laboratory implied that 2,4,5-T was teratogenic (producing malformed fetuses) in mice and rats. Subsequent studies have shown that a potential toxic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin, is responsible for the findings attributed to 2,4,5-T. The sample of 2,4,5-T employed in the Bionetics study contained 27 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Additional studies have shown that oral administration of 2,4,5-T containing < 1 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin produced no teratogenic effects on r rabbits or mice. The obvious concern is produce 2,4,5-T without the contamin

The 2,3,7,8-tetrachlorodibenzo-p-dic can be formed in the manufacture of precursor 2,4,5-trichlorophenol. The cor tions required for its formation are h temperatures and basic conditions. This occur in the alkaline hydrolysis of 1,2,4 tetrachlorobenzene to the trichlorophe

No detectable dioxins have been obser in 2,4-D. This is due to the fact that precursor 2,4-dichlorophenol is made direct chlorination of the phenol and not alkaline hydrolysis of 1,2,4-trichlorob zene.

To date analytical methods have be developed and validated for a method s sitivity of 0.5 ppm for 2,3,7,8-tetrachlo dibenzo-p-dioxin in 2,4,5-T acid. With pro manufacturing controls there is no probl in producing 2,4,5-T with no 2,3,7,8-tet chloro-p-dioxin as indicated by these a lytical methods.

THE FUTURE

By December 31, 1970 the Industry T Force on Phenoxy Herbicide Tolerances have furnished to FDA the supplemer residue data necessary for the continu evaluation of the petitions to establish ne gible residue tolerances for 2,4-D, 2,4,5 silvex and MCPA on the appropriate fo crops and meat and milk tissues. Regist tions (USDA) are expected to remain force.

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TABLE 2. Results from 90-day subacute feeding studies of various phenoxy herbicides in rats and dogs.

	Approximate No-Ill Effect Levels (Mg/Kg/Day)			
	2,4-D	2,4,5-T	Silvex	MCPA
Rats	30	30	5-10	16
Dogs	10	5	7	8-10

Data provided to date indicate no hazard and no significant residues in food crops. Toxicology data support the use claims. The proper manufacturing of 2,4,5-T will alleviate the problems associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin.

The USDA has recently stated that prohibiting the use of phenoxy herbicides "would cost the U. S. farmers an additional $290 million to maintain current agricultural production. In addition, farmers and their families would have to work 20 million more hours to control the weeds without these herbicides. For this extra labor, the farmers would obtain no additional income".

Several hundred thousands of dollars have been expended over the past several years to prove the safety of phenoxy herbicides to man and his environment. From a scientific base the phenoxy herbicides can contribute economically, efficiently, and safely in the future for the control of broadleaved weeds and brush on food crops, pasture, rangeland, and non-cropland areas as they have for over 20 years.

SIGNIFICANT CHRONOLOGICAL EVENTS SINCE PREPARATION OF MANUSCRIPT

As of January 23, 1971 several significant events have occurred.

(1) The ITFPHT submitted amendments for 2,4-D, silvex and MCPA petitions on December 22, 1970. The Environmental Protection Agency (EPA) acknowledged filing of these amendments December 30, 1970.

(2) The ITFPHT also refiled the 2,4,5-T petition on December 22, 1970 and EPA advised on December 31, 1970 a petition number had been reserved but they (EPA) wished to know which specific 2,4,5-T formulations were to be covered by the petition and for industry to identify the ingredients (active and inert) contained in these products.

(3) The EPA has also advised that a specific tolerance for phenoxies in grass is necessary; a tolerance in grass had not been requested by ITFPHT as it did not appear necessary. In compliance with the EPA request, a level of 300 ppm for all phenoxies in grass was requested on January 6, 1971 by ITFPHT.

(4) On January 11, 1971 Dow was informed that the advisory committee from the National Academy of Science had been appointed on November 2, 1970 to review the food crop cancellation of 2,4,5-T.

At this writing, Dow and Hercules had each been given an hour on February 1, 1971 to present information relative to the safety of 2,4,5-T as used on food crops.

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Acta pharmacol. et toxicol. 1971, 29, 81–86.

From the Department of Microbiology, Dental Faculty,
University of Oslo, Norway

The Herbicide 2,4-Dichlorophenoxyacetic Acid I: Effects on L Cells

By

Jan Kolberg, Kristen Helgeland, Jon Jonsen and Olav Tjeltveit

(Received May 19, 1970)

Abstract: The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on L 929 cells in monolayer cultures has been studied. It was found that 2,4-D in the range of 50 to 500 µg/ml had a dose dependent inhibitory effect on cell growth. With 350 and 500 µg/ml complete inhibition of growth occurred after about 24 hrs of incubation. On removal of 2,4-D a rapid resumption of cell multiplication took place. This occurred even after exposure to 500 µg 2,4-D/ml for 12 days. In the presence of 250 to 500 µg/ml, vacuoles, which stained with lipid soluble dyes, appeared in the cytoplasm. On prolonged incubation with the herbicide, these vacuoles disappeared.

Key-words: Herbicide – L cells.

2,4-dichlorophenoxyacetic acid (2,4-D) belongs to the phenoxy group of herbicides which at low concentrations induces growth responses similar to the plant hormone auxin. At higher concentrations of these herbicides an excessive, uncontrolled growth leading to the death of the plant is observed. This is believed to be based on an abnormal metabolism of RNA (HANSON & SLIFE 1969).

2,4-D is probably used to a greater extent than any other herbicide, and descriptions of its toxicity and hazards to man, domestic animals and wildlife have been summarized by WAY (1969). The acute toxicity following oral administration appears to be moderate. The LD50 values found for a number of experimental animals range between 300 and 800 mg 2,4-D/kg body weight. In a recent, yet unpublished, study commissioned by the National Cancer Institute (U. S. A.) this herbicide was labelled as being a potentially teratogenic compound needing further study (*Science* 1969, *166*, 977 news and comment).

Since 2,4-D might be a teratogenic agent, studies to elucidate the mechanism of action on mammalian cells is of importance. For this purpose, cell cultures provide a suitable tool. The only study in this field has recently been

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made by LI & JORDAN (1969) who found a transient growth inhibition in suspension cultures of L cells exposed to 5 and 10 µg/ml of an ester of 2,4-D.

The present paper describes the effect of different concentrations of 2,4-D Na-K salt on the growth and morphology of L 929 cells in monolayer cultures.

Materials and Methods

Cell culture techniques.

Monolayers of mouse fibroblasts, strain L 929 (SANFORD *et al.* 1948) were used. The cultures were grown in Eagle's Minimum Essential Medium supplemented with 10 % calf serum, streptomycin and penicillin. Plastic petri dishes, 60 × 15 mm (Falcon Plastic Inc.) were seeded with 0.4 × 10^6 cells suspended in 5 ml medium and incubated at 37° in a humidified atmosphere of CO_2 in air. After incubation for 24 hrs, the medium was replaced by test media containing 2,4-D. The media were renewed every third day.

In reversal experiments the 2,4-D containing medium was removed and, before adding the control medium, the cell layers were washed twice with 2 ml of the latter medium.

For growth measurements, the cultures were trypsinized and counted in a Bürker haemocytometer. Each point on the growth curves represents the mean of the counts from 2 cultures.

Cytochemical analysis of lipids were carried out with Oil Red 0 in 100 % isopropanol or Sudan Black B in 70 % ethanol and counterstaining with Harris haematoxylin and neutral red respectively. The cells were not fixed before staining.

Cultures cultivated in Sykes and Moore chambers, volume 0.7 ml (SYKES & MOORE 1960) were photographed at 2 frames/min. by means of a Reichert inverted phase-contrast microscope with a 16 mm Beaulieu camera loaded with Kodachrome II A film. To reverse the effect of 2,4-D, the chambers were perfused with 2.5 ml control medium.

Herbicide.

A stock solution containing 25 mg 2,4-D/ml was prepared by dissolving the acid (Eastman Organic Chemicals) in aqueous NaOH-KOH (0.1 N : 0.1 N) and adjusting the pH to 7.3 with HCl. The solution was sterilized by filtration through a 0.22 µ Millipore-membrane filter.

Results

On exposure of L-929 cells in monolayer cultures to 2,4-D in concentrations from 50 to 500 µg/ml, a dose dependent inhibition of growth was found (fig. 1). Complete inhibition was found after 24 hrs in the presence of 350 and 500 µg 2,4-D/ml. Cytopathogenic changes such as rounding up and detachment of the cells were not seen, however, in the treated cultures.

When 2,4-D was removed from the cultures after incubation in the presence of 500 µg/ml for 3 or 12 days, inhibition of growth ceased (fig. 2). On removal of 2,4-D after exposure for 3 days there was a rapid increase in the number of cells concomitant with a disappearence of the vacuoles, whereas after 12 days there was a lag period of about 24 hrs before cell

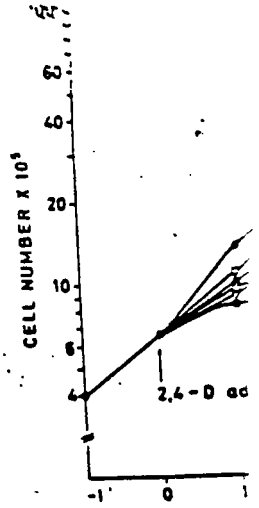


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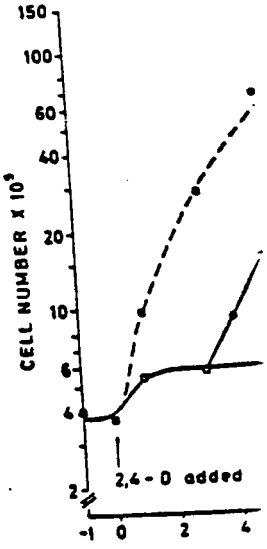


Fig. 2. Resu

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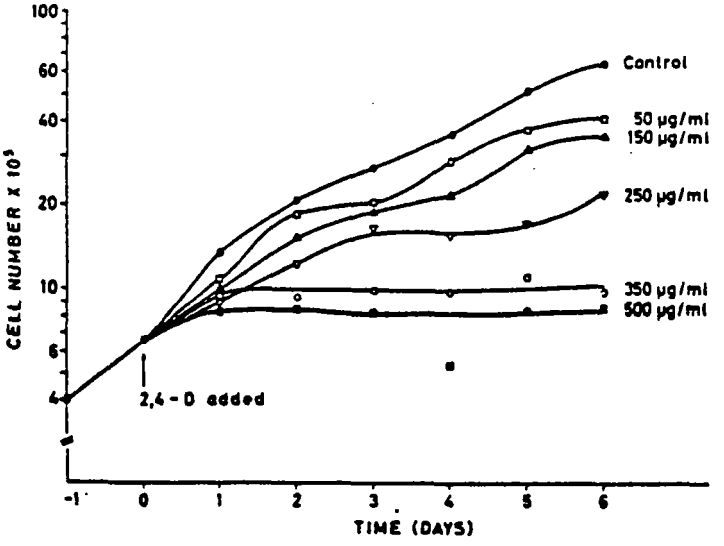


Fig. 1. The effect of various concentrations of 2,4-D on growth of L 929 cells.

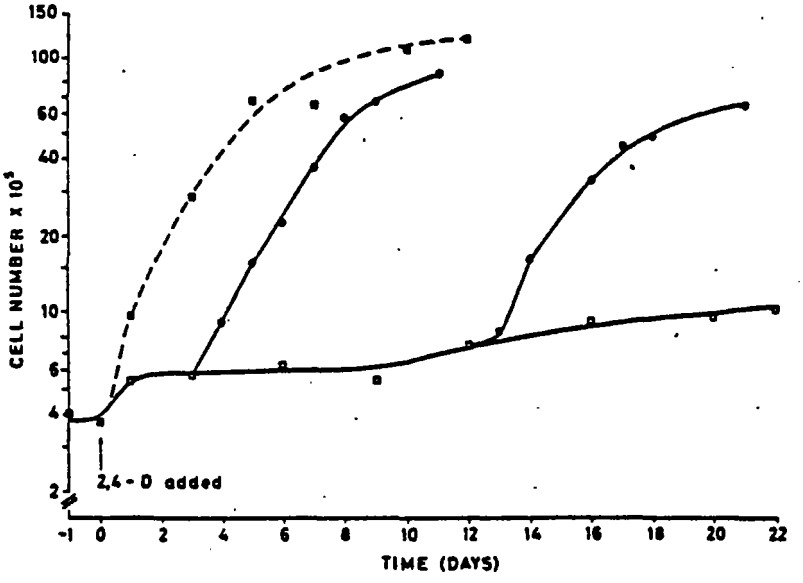


Fig. 2. Resumption of cell growth after removal of 2,4-D.
■ ---- ■ **untreated cultures.**
□ —— □ **cultures containing 500 μg/ml.**
● —— ● **cultures from which 2,4-D has been removed after 3 or 12 days of treatment.**

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Fig. 3. L 929 cells photographed under phase-contrast microscope.
a. control cells.
b. cells treated with 500 µg 2,4-D/ml for 30 hrs.
Magnification × 600.

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multiplication started. This experiment also shows that the cultures on prolonged incubation in the presence of 500 µg 2,4-D/ml exhibited an adaption to this compound and some growth occurred.

In the presence of 350 and 500 µg/ml, vacuoles of uniform size (fig. 3), which stained with Oil Red O and Sudan Black B, appeared in the cytoplasm after about 3 hrs of incubation, and their number increased during the next 21 hrs. On further incubation, the vacuoles disappeared, and after treatment for 4–5 days the cells were free from lipid-containing particles. At 250 µg/ml, vacuoles were observed after 24 hrs, but they disappeared within 2–3 days of incubation. No accumulation of vacuoles was found in the cultures treated with 50 and 150 µg/ml.

The observations made in the previous experiments were confirmed by time-lapse cinemicrography. During 33 hrs in the presence of 500 µg 2,4-D/ml no cell division was seen, and vacuoles were formed. When 2,4-D was removed a rapid resumption of cell growth took place, and the vacuoles disappeared within 22 hrs without being extruded intact into the medium. The cultures were followed for 32 hrs after removal of 2,4-D and there was no indication of a synchronizing effect from the treatment with this compound. No obvious effects of 2,4-D on pinocytosis and the undulating activity of the cell membranes were found.

Discussion

A concentration dependent inhibition of growth was found, when monolayers of L cells were exposed to 2,4-D. The inhibition was evident after 24 hrs, and with 350 and 500 µg 2,4-D/ml no net increase in the numbers of cells was found during the next 5 days of incubation. Some growth occurred, however, during the first 24 hrs in the presence of these two high concentrations which might indicate that 2,4-D was selectively toxic to a particular stage in the cell cycle. As this inhibitory effect of 2,4-D was readily reversible, a synchronizing effect would be expected if such a mechanism was operating. When cultures treated with 500 µg 2,4-D/ml for 33 hrs were transferred to control medium and examined with time-lapse cinemicrography, no burst of synchronized cell division was seen. This makes the above explanation unlikely.

The rapid resumption of cell growth on removal of 2,4-D after treatment for 3 and 12 days seems to exclude a state of unbalanced growth. This condition usually results in cell death on prolonged exposure to the inhibitor (MUELLER 1969).

During a period of 21 days, the L cells exhibited an adaption to growth in the presence of 500 µg/ml. This may be due to a selection of resistent cells and/or a prolongation of the generation time.

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It has previously been shown that changes in the extracellular environment cause accumulation of lipid-containing particles in cultured mammalian cells. These changes include a decrease in the extracellular pH, the addition to the medium of phenol or aliphatic polyalcohols, the replacement of horse serum by rabbit or human serum (MACKENZIE *et al.* 1967), and the addition of an unsaturated fatty acid such as oleate, linoleate and linolenate (MOSKOWITZ 1967). These vacuoles, like those induced by 2,4-D, stained with lipid soluble dyes and exhibited little or no tendency to fuse. However, these investigators found no growth inhibition with lipogenic factors in the medium, except for some growth retardation caused by the polyalcohols (MACKENZIE *et al.* 1968).

On continued incubation with 2,4-D, the vacuoles disappeared. MOSKOWITZ (1967) observed a similar phenomenon in oleate-treated L cells. He suggested that the particles might have been extruded intact into the medium by a secretory mechanism or, alternatively, that an intracytoplasmic hydrolysis might account for the breakdown and removal of the lipid particles. In favour of the hydrolytic mechanism, he demonstrated an increased lipase activity in steatotic cells. Whether a similar mechanism operates in our cells has not been investigated.

We found a transient lipid accumulation in 2,4-D treated cells and a release of growth after changing to the control medium. These findings suggest that the occurrence of lipid vacuoles in cell cultures is not necessarily indicative of degeneration.

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Acetic Acid

THE DOW CHEMICAL COMPANY

MIDLAND
February 19, 1971

DOW049272

H. L. Gordon, M.D. ✓
C. W. Hinman
J. E. Johnson
C. G. Kramer, M.D.
V. K. Rowe

Enclosed is a suggested protocol for the consumption of 2,4-D and 2,4,5-T by J. E. Johnson and C. W. Hinman. The protocol for the proposed study "Absorption and Excretion of 2,4-D and 2,4,5-T" to be conducted in India is also included. The latter protocol includes background toxicological information, metabolism data, and sample specifications. All of the information should be studied prior to conducting this study.

Since I have the samples of 2,4-D and 2,4,5-T, the study can be initiated whenever feasible. I suggest that the chosen guinea pigs and witnesses come to 1803 Building where the materials will be weighed out and consumed.

P. J. Gehring, D.V.M., Ph.D.
Chemical Biology Research
1803 Building
6-1089

Attachment

CONFIDENTIAL - SUBJECT TO INJUNCTION
D.C., E.D. MI. 4-4-78; DOW/EPA AGREEMENT 9-79

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PROTOCOL: HUMAN TOLERANCE OF 2,4-D AND 2,4,5-T

OBJECTIVE: To establish that the doses of 2,4-D and 2,4,5-T recommended for use in the study entitled "Absorption and Excretion of 2,4-D and 2,4,5-T In Man" are safe.

SUBJECT: J. E. Johnson- Director of Corporate Research and Development
C. W. Hinman- Director of Chemical Biology Research

COMPOUNDS:

Samples of 2,4-D (Sample AGR30653C) and 2,4,5-T (Sample AGR86187). These samples are analytical standards. The same materials are being used in the study "Absorption and Excretion of 2,4-D and 2,4,5-T In Man".

PROCEDURE:

A copy of the proposed study "Absorption and Excretion of 2,4-D and 2,4,5-T In Man" is attached. In the study proposed here, J. E. Johnson is to consume 81 mg of 2,4,5-T (1 mg/kg) and C. W. Hinman 79 mg 2,4-D (1 mg/kg). One week following this treatment, J. E. Johnson is to consume 405 mg (5 mg/kg) of 2,4,5-T and C. W. Hinman 395 mg (5 mg/kg) of 2,4-D. For administration, the compounds are to be mixed in a glass of milk. The milk is to be consumed in the presence of witnesses and a sworn affidavit obtained.

MEDICAL SURVEILLANCE:

The information provided in the accompanying protocol "Absorption and Excretion of 2,4-D and 2,4,5-T In Man" should be studied by Doctors Harold Gordon and Charles Kramer.

Prior to administration of the agents, it is to be ascertained whether there are any medical reasons for not using the chosen subjects. For a minimum of 24 hours following administration, a physician should be readily available. Physical examination prior to and following administration of the agents will be performed at the discretion of the physician involved.

ANALYTICAL WORK:

It is suggested that a 10 ml sample of blood be obtained at 2, 7, 12, 24 and 48 hours following administration of the 5 mg/k dose. These samples should be analyzed for 2,4-D and 2,4,5-T.

It is requested that all urine voided during the first and second 24 hour periods following administration be collected. A sample of these composite samples is to be analyzed for the agent in question.

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ABSORPTION AND EXCRETION OF 2,4-D AND OF 2,4,5-T IN MAN

The extensive use of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as selective herbicides in many areas of the world has increased the possibility of exposure of animals and man to these materials. This has created the need for greater knowledge and understanding of their fate after ingestion by man, as well as other animals. Such understanding is of great value in using data obtained from animal experimentation to assess safety for man.

DOW0492774

SUMMARY OF TOXICOLOGY LITERATURE

There are a number of reports in the literature in which the acute and chronic oral toxicity of 2,4-D and 2,4,5-T were determined for a variety of animal species. In 1947, Hill and Carlisle reported the acute oral toxicity of 2,4-D in mice, rats, rabbits, guinea pigs and monkeys. Of these species the monkey was most sensitive showing no adverse effects at 214 mg/kg dosages but definite signs of toxicity at 428 mg/kg. The toxic signs observed were nausea and vomiting, stiffness of the legs with some muscular incoordination, lethargy, hanging of the head and ptosis of the eyelids.

In 1948, Biorn and Northen reported the acute oral lethal dose of 2,4-D for chickens to be between 380-765 mg/kg. In 1953, Drill and Hiratzka reported on studies with dogs indicating the acute oral LD_{50} value for 2,4-D to be about 100 mg/kg. When 2, 5 or 10 mg/kg/day of 2,4-D was administered 5 times/week for 90 days, no adverse effects were noted; however, 20 mg/kg caused the death of 3 of 4 dogs on days 18, 25, and 49 of the test. The toxic signs which were observed varied from mild ataxia and stiffness of the hind legs to a definite myotonia, weakness, difficulty in swallowing, anorexia and occasional bleeding from the ears.

In 1954, Howe and Hymas reported the acute oral toxicity of 2,4-D to rats, mice, guinea pigs and chickens. The acute oral LD_{50} values and their 95% confidence limits were as follows: rats, [illegible] mg/kg (302-484); mice, 368 mg/kg (312-434); guinea pigs, [illegible] mg/kg (397-[illegible]); and chicks, 541 mg/kg (358-817). The toxicity incurred with repeated oral doses of 2,4-D in rats and

chicks was also reported. Rats fed 2,4-D five times a week for 4 weeks at levels of 3, 10, and 30 mg/kg/day showed no adverse effects of the treatment, whereas rats receiving levels of 100 mg/kg showed varying degrees of gastrointestinal irritation, slight cloudy swelling in the liver, and depressed growth rate. Animals receiving 300 mg/kg failed rapidly and died with severe gastrointestinal irritation being the principal effect observed.

In 1963, Palmer described the effects observed in cattle treated with varying amounts of 2,4-D five times/week for varying periods of time. A steer receiving 112 daily oral doses of 50 mg/kg showed no adverse effects. Another steer receiving 100 mg/kg/day developed a digestive disorder characterized by extreme tympany after 86 treatments. Another steer given 200 mg/kg/day developed muscular weakness in the hindquarters and a staggering gait after 34 days. A steer given 250 mg/kg/day developed moderate tympany and melena which was first noticed after 15 days on treatment.

A limited number of reports have appeared in the literature dealing with unusual exposure of humans to 2,4-D. In 1959, Goldstein, Jones and Brown reported 3 cases of exposures to an unspecified ester of 2,4-D. Severe sensory and motor symptoms necessitated hospitalization in each case. The disorders began some hours after the arms and/or legs of the patients had become wetted with unknown amounts of the 2,4-D preparation being used to kill weeds. The symptoms progressed for a period of days until pain, paraesthesia and paralysis were severe. Disability was protracted and recovery was incomplete even after a lapse of years. Electromyographic examination supported the diagnosis of peripheral neuropathy. In 1962, Todd described a patient with peripheral neuritis lasting almost two years which was presumably caused by the same agent. In 1963, Berkley and Magee reported the development of a neuropathy in a farmer following exposure to the dimethylamine salt of 2,4-D.

In 1965, Neilsen, Kaempe and Jensen-Holm reported the only known fatal case of 2,4-D poisoning. The suicidal patient had ingested approximately 200 mg/kg of 2,4-D but the actual lethal dose could not be established.

In 1970, Berwick reported on a farmer who accidentally ingested about 30 ml of a concentrated weedkiller formulation containing 6% 2,4-D isooctylester, 49% S-ethyldipropylthiocarbamate (Eptam), 9% kerosene, 0.5% epichlorohydrin and 5%

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emulsifiers. The amount of 2,4-D ingested was calculated to be 110 mg/kg as the free acid. In addition, 230 mg/kg of S-ethyldipropylthiocarbamate, and 2.3 mg/kg of epichlorohydrin must have been ingested. The patient was hospitalized and his progress through the acute stages and subsequent recovery were closely monitored. Symptoms associated with intoxication included nausea and acute gastritis, breathing difficulties, skeletal muscle injury and weakness. The levels of LDH, SGOT, SGPT, aldolase and creatine phosphokinase in the serum were elevated. No symptoms of peripheral neuropathy were detected. The patient was discharged from the hospital after 2 weeks. Laboratory studies one month after ingestion of the material showed normal values for the serum enzyme levels, the complete blood cell count and the urinalysis.

The most meaningful data relative to establishing the safety of the doses of 2,4-D suggested foruse in the present study were reported by Seabury in 1963. He reported on the administration of 2,4-D as a treatment for two terminal patients with disseminated coccidioidomycosis. One patient received 12.712 g of the sodium salt of 2,4-D during a period of 34 days without observable toxicity. The drug was administered both intramuscularly and intravenously. On the 34th day, 2 gm were given intravenously without detectable untoward effects. On the 36th day, a dose of 3.600 g of sodium 2,4-D was given intravenously over a period of 2 hours. Untoward effects associated with the administration of the latter dose were stupor, hyporeflexia, urinary incontinence and muscular weakness. Within 48 hours after treatment the patient had returned to his prereaction status and no further evidence of neurologic or muscular change appeared in the subsequent two weeks of his life.

Another study in which the dose of 2,4-D was accurately quantitaed was that of Assouly (1951). He ingested 500 mg/day for 3 weeks without experiencing adverse effects.

The survey of the literature revealed only limited information on the toxicity of 2,4,5-T to animals. No cases of unusual human exposures have been reported. In 1953, Drill and Hiratzka reported the acute oral LD_{50} of 2,4,5-T in dogs to be in the range of 100 mg/kg or higher. No marked toxic effects were noted but there was some wieght loss, anorexia, ataxia and slight to moderate stiffness in the hind legs in dogs receiving higher doses. Dogs receiving 2, 5 or 10 mg/kg of 2,4,5-T five days a

DOW0492276

week for 90 days showed no adverse effects. The daily administration of 20 mg/kg produced death in 4 dogs after 11, 49, 59 and 75 days of treatment. In the dogs that died, toxic signs were weakness, stiffness of hind legs, difficulty in swallowing food and bleeding from the gums.

DOW040277

In 1954, Rowe and Hymas reported the acute oral toxicity of 2,4,5-T in rats, mice, guinea pigs and chickens. The acute oral LD_{50} values and the 95% confidence limits were 500 mg/kg (391-640), 389 mg/kg (245-619), 381 mg/kg (307-472) and 310 mg/kg (211-456), respectively. The toxic signs at the higher levels of treatment were anorexia, weight loss, depression, rough hair coat and muscular weakness.

Recently, a study of the effects of 2,4,5-T consumption for 90 days has been conducted using male and female rats. (Study conducted during 1970 by The Dow Chemical Company, Midland, Michigan and as yet unpublished.) In this study, 2,4,5-T was included in the diet such that daily doses of 100, 30, 10 and 3 mg/kg/day were given to groups of male and female rats, 10 animals/group. Parameters evluated were appearance, behavior, growth, food intake, hematology, urinalysis during the 12th week of the experiment, terminal determination of blood urea nitrogen (BUN), serum alkaline phosphatase and SGPT, terminal organ weights and gross pathological and histopathological examination of the tissues. There was 100% survival in all groups. At the 100 mg/kg/day level the following signs of toxicity were observed: depressed body weight, depressed food intake, a two-fold increase in serum alkaline phosphatase, a 20% increase in SGPT (observed only in males), inconsistent paleness and accentuated lobular patterns in the liver of some rats, and cloudy swelling of the hepatocytes. At the 30 mg/kg/day level, the only changes associated with treatment were increased liver and kidney weights in males and a slight elevation of serum alkaline phosphatase and SGPT in the females. No changes were detected in rats given 10 or 3 mg/kg/day.

Studies designed to reveal whether 2,4,5-T is teratogenic have been conducted by The Dow Chemical Company, Zionsville, Indiana (In Press.) In these studies, rats were given 1, 3, 6, 12 or 24 mg/kg/day 2,4,5-T as a 0.25% suspension in METHOCEL by oral gavage on days 6 through 15 of gestation and rabbits were given 10, 20 or 40 mg/kg/day orally in gelatin capsules on days 6 through 18 of gestation. At the doses indicated, no untoward effects were detected in either the mothers or the fetuses.

PHARMACOLOGY OF 2,4-D AND 2,4,5-T

Absorption and Distribution

Orally administered, 2,4-D or 2,4,5-T is rapidly absorbed from the stomach and distributed to all tissues. The compounds have been detected in blood and organs as early as 1 hour after dosing, with peak levels occurring between 4 and 8 hours after administration in several mammalian species (Khanna and Fang, 1966; Shafik et al, 1971; Erne, 1966; Courtney, 1970). The greatest concentrations occur in plasma, liver, kidney and lung.

DOW 049278

Metabolism and Excretion

2,4-D and 2,4,5-T have been shown to be eliminated completely and intact in urine of cattle (Lisk et al, 1963; Bache et al, 1964; St. John et al, 1964) and sheep (Clark et al, 1964). Courtney (1970) reported that greater than 90% of an administered dose of 2,4,5-T to rats was recovered unchanged within 72 hours after treatment. She suggested that 2,4,5-T was apparently not metabolized to any extent in the rat. However, Shafik et al (1971) found that treatment of rats with 50 mg/kg 2,4,5-T resulted in the excretion of at least three metabolites in addition to the parent compound. In the same study, rats dosed with 2,4-D excreted the major portion of the administered herbicide in the urine. No metabolites of 2,4-D were detected. Khanna and Fang (1966) administered 1-C^{14}-2,4-D to rats and found no C^{14} in expired air during a 3-day period following dosing. In the latter study counter-current separation of urine and tissue extracts revealed a very small quantity of an unidentified metabolite(s) (0.25% of total radioactivity in urine).

The excretion rate of 2,4-D and 2,4,5-T appears to be somewhat dependent upon the dose administered. High dose levels (>250 mg/kg) are excreted at a slower rate than low dose levels (Khanna and Fang, 1966; Shafik, et al, 1971). The plasma half-life values are about 3 hours for rats, 8 hours for calves and chickens and 12 hours for pigs (Erne, 1966). The major excretory route is via the kidneys in all mammalian species studies. Following administration of a tracer dose of C^{14}-2,4-D to sheep, 96% of the activity was excreted in the urine and 1.4% in the feces in 72 hours (Clark et al, 1964). Only low levels of 2,4-D were found in feces of rats and pigs and in bile of pigs given 2,4-D orally (Erne, 1966). Renal excretion of

phenoxyacetic acids has been reported to be the predominant mode of excretion in cattle and sheep (Lisk et al, 1963; Bache et al, 1964 and St. John et al, 1964). Shafik et al (1971) found that in rats, orally administered 2,4-D and 2,4,5-T were almost completely excreted in the urine within 2 days. Khanna and Fang (1966) reported 94 to 99% of 2,4-D is excreted in urine and feces of rats within 48 hours following the administration of 1-10 mg 2,4-D per rat. Of this, 93 to 96% was excreted in the first 24 hours; almost all was excreted in the urine.

DOW 04982/79

EVALUATION OF THE SAFETY OF THE PROPOSED STUDY ON MAN

Acute and chronic toxicological studies on various animal species (absorption, distribution and excretion) indicate that there is no significant difference in either the toxicity or metabolism of 2,4-D and 2,4,5-T. In the most susceptible animal, the dog, the LD_{50} for a single dose of either 2,4-D or 2,4,5-T is at least 100 mg/kg. In this specie, a dose of 10 mg/kg/day of either compound is without adverse effect when given 5 times a week for 3 months. Data from various studies on other species show all of them to be less sensitive than the dog.

Reports of accidental ingestion and therapeutic administration of 2,4-D to man strongly suggest that man is not uniquely sensitive to 2,4-D in comparison to other species. Based on the similarity in biological effects of 2,4-D and 2,4,5-T in animals, it seems reasonable to expect 2,4,5-T to be similar to 2,4-D in its effect on man.

Thus, based on those experiences in which the ingestion of 2,4-D by man was quantitated and on the biological similarity of 2,4-D and 2,4,5-T in animals, a single oral dose of 5 mg/kg of either 2,4-D or 2,4,5-T is believed to be safe for man.

PRE-EXPERIMENTAL SAFETY CHECK

Although the available data suggest that a dose of 5 mg/kg 2,4-D and 2,4,5-T will not cause any untoward effects in man, it would be prudent to establish the subjective tolerance of this dose before conducting the experiment. Therefore, it is recommended that two test subjects per material be given 1 mg/kg 2,4-D and 2,4,5-T. When it is established that this dose does not produce untoward effects, the dose should be sequentially increased to 3 mg/kg and then to 5 mg/kg. If the experimentalists believe that selected clinical studies such as those proposed

(Paragraph 6 of Experimental Design) are desirable, they should, of course, conduct them. The test subjects used for this study should not be used in the absorption and excretion experiment.

EXPERIMENTAL DESIGN

1. Subjects: Five male volunteers between the ages of 20 and 40 years will be used for each compound. Prior to exposure, the individual will be examined and judged to be of normal health and nutritional status and should not be on medication. Clinical parameters to be assessed at this time and during the experiment are indicated below.

2. Samples of 2,4-D (Sample AGR30653C) and 2,4,5-T (Sample AGR86187). The samples of these material will be analytical standards supplied by Dow. Specifications for these samples are included.

3. Administration: 2,4-D and 2,4,5-T will be given in a single dose of 5 mg/kg in a gelatin capsule. The test material is to be given one-half hour after a standardized light breakfast. Further meals for the first 24 hours should also be standardized.

4. Sampling Schedule
   a. Blood: Blood samples should be obtained as indicated below.

Time	2,4-D or 2,4,5-T Analysis	Clinical Chemistry
0 (Pretreatment)	20 ml	30 ml
2 Hours post-treatment	10	-
5 "	10	-
8 "	10	-
12 "	10	20
16 "	10	-
24 "	20	-
48 "	20	20
168 "	20	20

The blood samples for analysis of 2,4-D and 2,4,5-T are to be mixed with 2 mg potassium oxalate per ml of blood and centrifuged. Plasma is to be collected for compound analysis. Duplicate analysis should be made if possible.

DOW 049280

The blood samples for clinical chemistry should be collected without anticoagulant. The clinical chemistry determinations will be made on serum.

b. Urine: A pretreatment 24-hour urine sample is to be collected as a control. Following treatment, all urine is to be collected. During the first 24 hours, the volume of the urine voided and the time following treatment at which it was voided should be recorded. A 50 ml aliquot of each urine sample is to be taken for compound analysis. Clinical chemical evaluations are to be conducted on another aliquot. After the first 24 hours, a composite urine sample for each subsequent 24-hour period is to be collected and an aliquot, 100-200 ml, retained for compound analysis.

c. Feces: All feces voided on each of the 7 days following treatment should be collected. The time collected and the weight of each sample is to be determined. A representative aliquot of the feces, 100 to 200 gm, is to be retained for analysis.

(All samples - plasma, urine, feces - are to be refrigerated as soon as possible after collection and prior to analysis. The analytical procedures to be used will be submitted with the protocol.)

5. Analysis: The procedures for the analysis of 2,4-D and 2,4,5-T are included. Prior to attempting analysis of specimens obtained from test subjects, known amounts of 2,4-D and 2,4,5-T should be added to plasma, feces and urine and the percent recovery should be established. Analysis of samples obtained from the test subjects should not be conducted until a recovery of greater than 90% has been established. This is necessary to provide a basis for the evaluation of the validity of the analytical methods in the hands of the experimentalist.

The evaluation of results from the first test subject for each compound should be completed before using subsequent test subjects to confirm that the dose was appropriate and the sampling times were appropriate. Adjustments can be made as deemed necessary.

DOW 0135

6. Physical and Clinical Evaluation of Subjects: In addition to obtaining a history, a thorough physical examination, including a neurological evaluation, will be conducted on each subject. Any evidence of abnormal nutrition, gastrointestinal, urinary, cardiac, muscular, or neurological conditions will exempt the individual from use in this study.

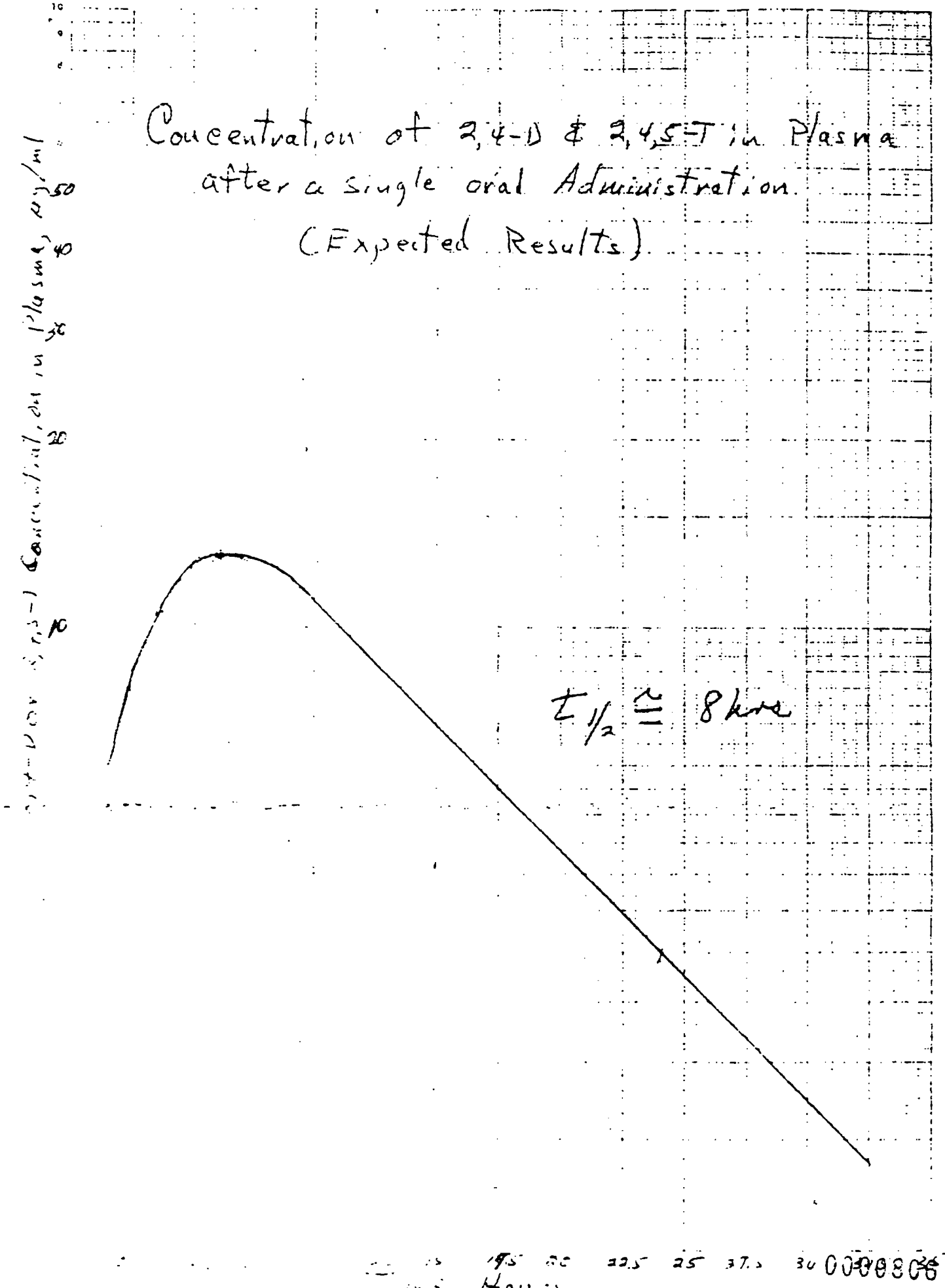
If currently feasible, the clinical tests should include the following: electrocardiogram, electroencephalogram, electromyogram, urinalysis (color, transparency, specific gravity, pH and concentrations of protein, glucose, acetone, myoglobin, blood, bile, as well as microscopic examination of the sediment , SGPT, SGOT, serum LDH and component isozymes, serum aldolase and serum creatine phosphokinase. Renal function is to be evaluated by determining the clearance of PAH and creatinine. Hematological evaluation is to include a complete blood count and hemoglobin determination.

In addition to the clinical evaluation prior to treatment, it should be repeated 12, 48 and 168 hours following the administration of the test compound.

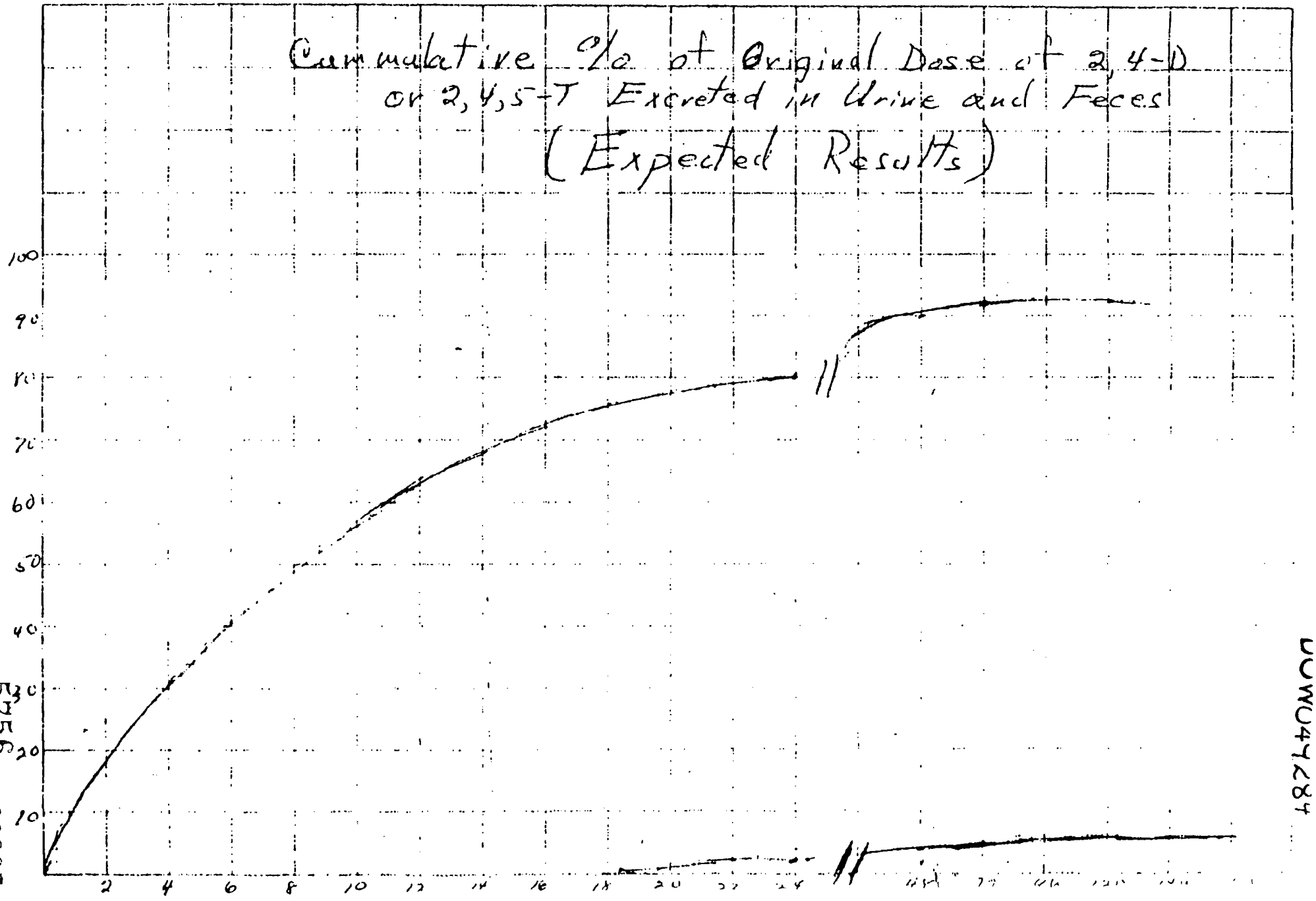
7. Expected Data: Examples of the character of the data to be obtained from these experiments are shown in the attached hypothetical figures. Once the data have been obtained, we have a computer program which will allow us to determine the rate of absorption, the rate of disappearance and the volume of distribution for each experimental subject. These values will then be subjected to a statistical evaluation to determine the mean and standard deviation.

CONFIDENTIAL

Concentration of 2,4-D & 2,4,5-T in Plasma after a single oral Administration. (Expected Results)



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UUW047284

THE DOW CHEMICAL COMPANY
MIDLAND MICHIGAN

ANALYTICAL LABORATORIES

REPORT

DATE October 13, 1969 CHARGE 1263-026

AL NUMBER 7-932 PROBLEM 5602372

YOUR NUMBER

DESCRIPTION FULL DESCRIPTION OF ANALYTICAL STANDARD 2,4-D (2,4-DICHLOROPHENOXYACETIC ACID)

DOW 049285

Analytical standard 2,4-D, AGR 30653C, was found to be 99.7% pure. Identity was confirmed by data obtained by elemental analysis, mass spectrometry and nuclear magnetic resonance spectrometry.

1. Specifications for analytical standard 2,4-D: (Batches not meeting this specification will be reworked until they do or will be discarded).

	% Minimum	% Maximum
2,4-D	99.0	100.0
Volatile Impurities (100°C)	0.0	0.5
Impurities determined by Differential Scanning Calorimetry	0.0	1.0

2. Identity confirmed by:
   (a) Elemental Analysis (±0.3%):

Carbon	43.2	43.8
Hydrogen	2.45	3.05
Chlorine	31.8	32.4

   (b) Mass Spectrometry
   (c) Proton Nuclear Magnetic Resonance Spectrometry

3. Purity of the analytical standard will be determined as follows:
   (a) The purity of the analytical standard will be calculated as follows:

$$\% \text{ 2,4-D} = 100.0 - A - B$$

Where A = % volatile impurities
B = % imparities determined by differential scanning calorimetry

   (b) Determine volatile impurities by the following procedure:

SPLE. 1 HRS 23 OCT. 14

SIGNED R. Humnel

Approved by:

R. Humnel

AR 823

PHONE ME 6- 3543 BLDG. 574

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Weigh one gram of the standard into a dish and place the dish in a 100°C oven for 30 minutes. Cool the dish in a desiccator and reweigh. Calculate the percent volatile impurities from the loss in weight.

(c) Determine impurities in the dried analytical standard by analysis of the melting curve obtained with a Perkin-Elmer Differential Scanning Calorimeter:

(1) Accurately weigh three mg of the dried analytical standard into a sample pan and obtain its melting curve by heating in the calorimeter through a range of 405 to 420°K using a chart range of 2 mcal./sec., a heating rate of 0.625°C/min. and a chart speed of one inch per minute.

(2) Calculate the mole percent impurities in the sample from the melting curve following the directions given in Anal. Chem. 41, 330 (1969).

4. The identity of the analytical standard will be confirmed by the following procedures:

a. Elemental Analysis:

(1) Determine carbon and hydrogen by "Rapid Multiple Microdetermination of Carbon and Hydrogen", Mikrochimica Acta 1964, 631, or equivalent.

(2) Determine chlorine by method MLS. 66.1 (semi-micro oxygen flask combustion, potentiometric titration) or equivalent.

b. Mass Spectrometry:

The mass spectrum obtained with a suitable spectrometer should show:

(1) A parent ion at mass 220 with isotope peaks indicating the presence of two chlorine atoms.

(2) A fragment ion at mass 175 (loss of -COOH).

(3) Fragment ions at mass 162 and 161 (loss of -CHCOOH and $-CH_2COOH$).

(4) A fragment ion at mass 145 (loss of $-OCH_2COOH$).

c. Proton Nuclear Magnetic Resonance Spectrometry:

The 60 MHz proton spectrum obtained by scanning a 20% solution of the analytical standard in hexadeuterated acetone with tetramethylsilane as internal standard should show:

(1) A singlet at -4.82 ppm (methylene protons in $-OCH_2-$).

(2) A doublet at -7.04 ppm, J = 8.8 Hz, a doublet, J = 8.8 Hz, of doublets, J = 2.5 Hz, at -7.26 ppm and a doublet at -7.42 ppm, J = 2.5 Hz. (Chemical shifts and coupling constants characteristic of a 2,4-disubstituted phenoxy ring).

(3) A singlet at -9.15 ppm due to an exchangable proton, (carboxylic acid).

(4) Integrals of the ratio: 2 methoxy protons: 3 aromatic protons: one acid proton.

DOW049286

5. Synthesis, purity and identity of the 2,4-D standard now in use (July 17, 1969). Purity and identity established by methods in sections 3 and 4 respectively.

a. Synthesis and purification of analytical standard 2,4-D, AGR 30653C:

Technical grade 2,4-dichlorophenoxyacetic acid was purified by dissolving in sodium hydroxide solution, precipitating with acid, washing with water and drying.

b. Tests for purity of analytical standard 2,4-D, AGR 30653C:

2,4-D	99.7%
Volatile Impurities	0.20%
Impurities determined by Differential Scanning Calorimetry	0.06%

c. Tests for identity of analytical standard 2,4-D, AGR 30653C:

(1) Elemental Analysis:

(a) Carbon	43.8%
(b) Hydrogen	2.9%
(c) Chlorine	31.8%

(2) Mass Spectrometry:

The mass spectrum showed:

(a) A parent ion at mass 220 with isotope peaks indicating the presence of two chlorine atoms.

(b) A fragment ion at mass 175.

(c) Fragment ions at mass 162 and 161.

(d) A fragment ion at mass 145.

(3) Proton Nuclear Magnetic Resonance Spectrometry:

The proton spectrum showed:

(a) A singlet at -4.82 ppm (methylene protons).

(b) A doublet at -7.04 ppm, J = 8.8 Hz, a doublet, J = 8.8 Hz, of doublets, J = 2.5 Hz, at -7.26 ppm and a doublet at - 7.42 ppm, J = 2.5 Hz, (aromatic protons).

(c) A singlet at -9.15 ppm due to an exchangable proton (acid proton).

(d) Integrals of the ratio: 2 methylene protons: 3 aromatic protons: 1 acid proton.

MON 049287

References

Proof of Structure:

Chemical-Physics Research Lab. combined report IR 203894, MS 205431, NMR 205275 by S. T. King, L. Shadoff and T. E. Evans.

Elemental Analysis:

Analytical Laboratories AL 9-150, J. Simpson, C. Mendoza.

Preparation of Analytical Standard:

L. White, Process and Formulations Research Lab.

DOW 0193248

Additional Analytical Work on Sample AGR 30653C

It was necessary to dry the 2,4-D standard in order to obtain a meaningful purity result with the differential scanning calorimeter. Without drying a result of 99.0 mole percent was obtained. Karl Fischer determination of water in the standard showed that the drying loss was due to water (0.19% water, AL 9-645, R. A. Dutcher). To confirm the suitability of the calorimetric purity determination 2,6-D was added to a portion of the standard and satisfactorily detected using the differential scanning calorimeter.

A 0.4% unknown found on gas chromatographic analysis after methylation with diazomethane is thought to be a result of the diazomethane reaction as it is found in about the same amount in all phenoxyacids methylated in this way (AL 5-666, J. Russell). This unknown was not found after esterification with methanol-sulfuric acid.

ajg

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By Dr. Julius E. Johnson
Vice-President and Director of Research
The Dow Chemical Company

Safety in the Development of Herbicides

The consideration of this broad topic can well be divided into three parts—past, present and future.

The portion entitled "past" will deal primarily with 2,4,5-T and other phenoxy herbicides. The chapter on the "present" will give you a portrait of the events *currently* involved in establishing the safety of a new pesticide. The concluding remarks on the "future" will present some of my opinions on what is needed to encourage and sustain a healthy weed control enterprise for the future.

Past

A controversy surrounding 2,4,5-T emerged on October 29, 1969. The results of a National Cancer Institute study on teratology conducted by the Bionetics Laboratory were made known through an announcement by the President's Science Advisor, Dr. Lee Dubridge. Immediately thereafter I was personally involved in reporting to the Government the possibility that the sample used in the Bionetics test was contaminated. The suspected contaminant was 2,3,7,8-tetrachlorodibenzo-p-dioxin. These suspicions were later confirmed by analysis of the Bionetics sample. Efforts were immediately instituted to test the effect of commercial 2,4,5-T containing less than 1 part per million of the contaminant. Tests were conducted in Dow laboratories after thorough discussions with government scientists concerning the methods to be used. Sprague-Dawley rats were the recommended test animals. Repeated daily oral administration of 2,4,5-T by stomach tube to the pregnant rats was the recommended route of administration. The first series of dosages ranged up to 24 milligrams per kilogram body weight, the second series to 100 milligrams per kilogram. None of these dosages produced the symptoms of teratology claimed in the Bionetics report. The tetrachlorodibenzo-p-dioxin, on the other hand, was toxic to embryos at a dosage level of 0.125 micrograms per kilogram per day; 0.03 micrograms per kilogram was *below the no-effect level.* Only by combining 2,4,5-T at a dose of 50 milligrams per kilogram with the tetrachlorodibenzo-p-dioxin in a third experiment, was it possible to produce cleft palate in a few of the test rats. (Figure 1). This is as close as we were able to come confirming the Bionetics work in rats. Later tests with 2,4-D and silvex also failed to produce terata at elevated dosages.

It is noteworthy that the tests which were conducted by Bionetics Laboratories and by the National Institute of Environmental Health Sciences that were interpreted as showing 2,4,5-T to be teratogenic were administered by subcutaneous injections of 2,4,5-T dissolved in dimethylsulfoxide. The doses were up to 113 milligrams per kilo-

5762

FIGURE 1—Teratology Study in Rats: 2,3,7,8-tetrachlorodibenzo-p-dioxin Added to 50 MG pure 2,4,5-T/KG Body Wt./Day.

Treatment	No. of Litters	No. of Fetuses	Litters with Cleft Palate	Litters with Intstnl. Hemorr.
Control (Vehicle)	17	194	0	0
50 mg Pure 2,4,5-T/kg	17	172	0	0
+0.01 μg TCDBD	15	150	0	0
+0.03 μg TCDBD	17	173	0	0
+0.06 μg TCDBD	17	155	1	8
+0.125 μg TCDBD	15	134	0	11
+0.5 μg TCDBD	14	76	4	13
+1.0 μg TCDBD	15	46	5	8

FIGURE 2—Acute Oral Toxicity, Single Oral Dose.

	LD_{50} as mg/kg Body Weight	
	Rat	Guinea P
2,4-D	375.0-666.0*	1,000.0
2,4,5-T	500.0	380.0
Silvex	500.0	650.0
Picloram	8,200.0	3,000.0
DDT	150.0-800.0*	400.0
Parathion	1.7-30.0*	9.3-32.0
2,3,7,8-Tetrachloro-dibenzo-p-dioxin	0.022-0.045*	0.0006

*Varies according to vehicle or sex

gram body weight in the Bionetics test[1], and up to 100 milligrams per kilogram in the NIEHS tests[2].

These procedures are not at all relevant to actual exposures and it is questionable whether the demonstration of teratogenicity by these methods is meaningful. Golberg[3] has recently pointed out several complications worthy of citation—

"In checking on potential teratogenicity of trace chemical contaminants in food, the height of absurdity is achieved by the combination of a maximum tolerated dose, a parenteral route of administration, and the application of zero tolerance on the basis of the results. In this area we know full well that any one of a host of adverse influences on the mother is reflected in fetal deaths, resorptions and/or abnormalities. Teratogenic effects are elicited by: transport of mice by air on days 12 and 13 of pregnancy (Brown, Johnston & Niswander, 1970): fasting for 24 hr. or less at a critical stage of gestation (Kalter & Warkany, 1959) which may be brought about inadvertently or unsuspectingly by inducing somnolence, lethargy, muscle weakness or ataxia; a diet of raisins for one day (Peters & Strassburg, 1969): severe limitation of movement or avoidance behavior (Rosenzweig & Blaustein, 1970): and many other nonspecific factors probably acting through a stress mechanism, as well as hyper- or hypothermia and endocrine influence (Kalter & Warkany, 1959). Even subcutaneous sodium chloride is teratogenic in mice (Nishimura & Miyamoto, 1969).

Here, above all, is a situation that demands the utmost care in selecting doses that do not render the mother sufficiently ill to produce even transient inappetence. The use of a maximum tolerated dose overlooks the possibility of non-specific toxic stress . . ."

There was ample justification for con by government officials over the results covered with the original sample use Bionetics. This sample, however, (colle in 1964) was not typical of carefully duced 2,4,5-T. 2,3,7,8-tetrachlorodibe p-dioxin is an extremely toxic subst (Figure 2). This is good and sufficient rea to maintain the tetrachlorodibenzo-p-di concentration as low as possible. With analytical methods and production t niques available in 1965 a one part per lion level was a realistic specification. has since been reduced to 0.5 parts million.

A few comments on the characteristic 2,3,7,8-tetrachlorodibenzo-p-dioxin will of interest. This compound can be for during the manufacture of 2,4,5-trichl phenol where two moles condense to f a dioxin (Figure 3).

The following information is known ab the stability and properties of the 2,3,7 tetrachlorodibenzo-p-dioxin:

(a) in solution it is rapidly degraded by traviolet light at wavelengths that pear in the spectrum of the s (Figure 4).

(b) The tetrachlorodibenzo-p-dioxin one-fifth the solubility of DDT in wa and one-sixteen hundredth the so bility of DDT in benzene (Figure Thus, there is probably less tender to concentrate in fat, but the quest is really academic because the quan ties of tetrachlorodibenzo-p-dioxin the environment are exceedingly sm

Concern has been expressed that the co bustion of 2,4,5-T (even though the para material contained no tetrachlorodibenzo dioxin) would cause dioxin formation. laboratory experiment was conducte wherein agent orange (a defoliant contai ing equal quantities of the n-butyl esters 2,4-D and 2,4,5-T) was applied to 18.5 c

5763

ter paper at rate of 24 lb. per acre (Figre 6). This is equal to approximately 10 lb. 2,4,5-T acid equivalent per acre. The aper was burned and combustion products us air drawn through an absorber packed ith glass beads cooled with liquid nitroen. The combustion products plus the ash as solvent extracted and analyzed by gas hromatography. The burning temperatures ncountered are shown in Figure 7. There as no 2,3,7,8-tetrachlorodibenzo-p-dioxin etected in the combusion products at a ensitivity of 5 ppm. A similar experiment as tried using wood as a substrate but here were substances in the combustion roducts which interfered with the analyis. Speculative claims are widespread that 2,4,5-T residues on vegetation might be converted to 2,3,7,8-tetrachlorodibenzo-pdioxin if the dead foliage is burned. All available evidence to date indicates this conversion does not occur. The alleged precursor is in dilute form on the substrate, the reaction is bimolecular—the molecules must be forced close together to react.

burning

Concern has also been expressed that traces of preformed tetrachlorodibenzo-p-dioxin contaminating 2,4,5-T sprayed into the environment would accumulate. The evidence to date is skimpy but nonetheless worth mentioning. C^{14}-labeled 2,3,7,8-tetrachlorodibenzo-p-dioxin dispersed on clay loam containing 3 percent organic matter gave off a very small amount of $C^{14}CO_2$ in two weeks when incubated at 24°C. (75°F.). This evidence suggests that degradation occurs slowly but it will require further observations to quantify the rate.

Kearney[4] has reported that the 2,3,7,8-tetrachlorodibenzo-p-dioxin is quite immobile in soil. Even in sandy soil there is little tendency to leach into the soil profile. Although persistent in soil the dioxin is not readily taken up and transported to the aerial portion of the plant.

Silvex is also made with 2,4,5-trichlorophenol as a starting material. A total of 8 *lots of silvex have been analyzed, obtained* from our 1967, 1968 and 1969 production. No detectable amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin have been found using a method having a sensitivity of 0.1 ppm. Before leaving the subject of contaminating chlorodibenzo-p-dioxins it is noteworthy that no detectable dibenzo-p-dioxins (including the 2,3,7,8-tetrachloro- or the 2,7-dichloro-) have been found in 2,4-D samples analyzed. The sensitivity of *the analytical method used was 1 ppm.*

The formation of 2,7-dichlorodibenzo-p-dioxin does not occur in the manufacture of 2,4-dichlorophenol since it is made by direct chlorination of phenol and not by alkaline hydrolysis of a trichlorobenzene. Re-

FIGURE 3—Formation of 2,3,7,8-tetrachlorodibenzo-p-dioxin.



FIGURE 4—Effect of radiation from G.E. Sunlamp at distance of one meter on 12 ppm solution of 2,3,7,8-tetrachlorodibenzo-p-dioxin.

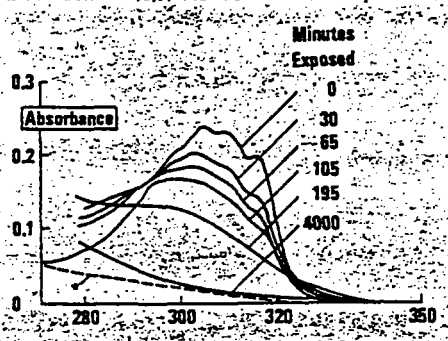


FIGURE 5—Comparative solubilities, TCDBD vs DDT.

Solvent	Solubility at 25°C TCDBD	DDT
Water	0.2 ppb	1 ppb
Benzene	0.047 g/100 ml	78 g/100

FIGURE 6—Burning Agent Orange Applied to Filter Paper.

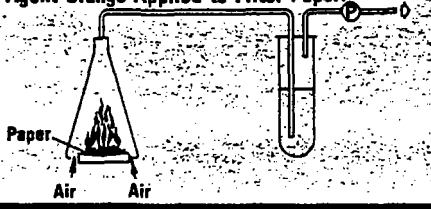


FIGURE 7—Combustion of Agent Orange on Filter Paper.

	°F	°C
Paper Surface	600-800	300-40
Body of Flame	1000-1200	550-65
Front of Flame	1300-1400	700-75

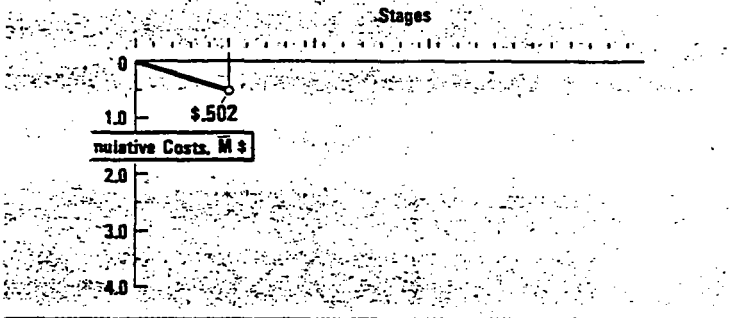
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URE 8—Safety Evaluation For New Pesticide—Minimum Registration Requirements.

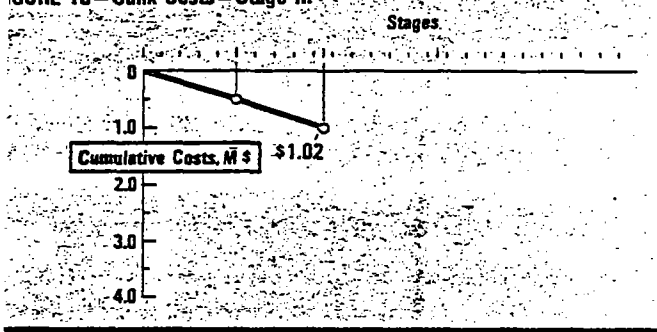
	1950	1960	1970
	Acute, Lab. Animals	Acute, Lab. Animals	Acute, Lab. Animals
KICOLOGY	30-90 day, rat	90 day, rat 90 day, dog 2 year, rat 1 year, dog	90 day, rat 90 day, dog 2 year, rat 2 year, dog Reproduction, 3 gen., rat Teratogenesis, rodent Fish, shellfish, etc. Birds
TABOLISM		Animal (min.)	Rodent, and/or dog Plant
ALYTICAL	Food crops, 1 ppm*	Food crops 0.1 ppm** Meat 0.1 ppm Milk 0.1 ppm	Food crops 0.01-0.05 ppm** Meat 0.1 ppm Milk 0.005-0.05 ppm
OLOGY			ENVIRONMENTAL Stability Movement Spectrum Accumulation

esticide only ** Pesticide plus toxic metabolite(s)

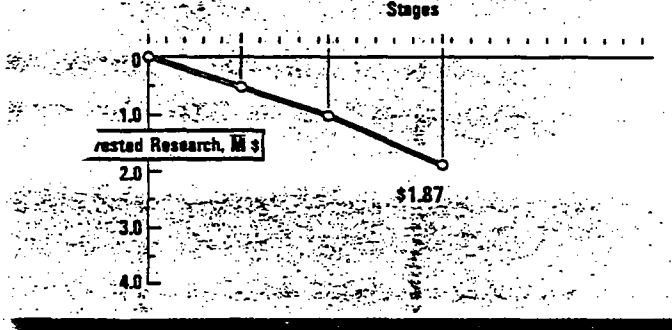
GURE 9—Sunk Costs—Stage I.



IGURE 10—Sunk Costs—Stage II.



IGURE 11—Sunk Costs—Stage III.



action conditions do not favor bimolecular condensation to form a chlorodibenzo-p-dioxin. Moreover, 2,7-dichlorodibenzo-p-dioxin is more difficult to form than the 2,3,7,8 - tetrachlorodibenzo - p - dioxin even when reaction conditions are favorable.

I would conclude these remarks by stating the firmly held opinion that commercially produced 2,4,5-T containing less than one part per million of 2,3,7,8-tetrachlorodibenzo-p-dioxin does not present a hazard to health. It is unfortunate that tests on one impure sample coupled with emotional concern over the use of 2,4,5-T as a defoliant in Vietnam have created unwarranted public fear concerning the appropriate use of this valuable herbicide.

Present

Let me now turn to the present and describe some of the experiments primarily relating to safety as they occur in the development of a new pesticide. For the purposes of this discussion we could substitute the word herbicide for pesticide because most of the safety considerations involve handling hazards, residues, non-target organisms and environmental considerations. I will briefly describe the stages through which a candidate progresses and then present some of the safety and cost considerations in each respective stage.

In a study reported by Von Rumker, Guest and Upholt[5], it was claimed, in 1969, in a survey of 13 companies that one compound out of 5,000 tested emerged as a marketable product. Testing required approximately five years prior to marketing and a cost of approximately four million dollars. This reported cost did not include process development or pilot plant studies. I would estimate today that one new pesticide emerges per 10,000 tested. The time from discovery to market ranges from eight to ten years and the cost is ten million dollars or more. This cost includes all research, development and technical service, process development, and pilot plant costs. It includes losers as well as winners. Two other research-intensive companies recently surveyed have privately given comparable estimates to us.

The challenge is to identify and eliminate the losers early. Conversely, the expensive way out is to have the second best hand in a poker game. You may be interested in comparing the minimum toxicological requirements for registration in 1950, 1960 and 1970 respectively (Figure 8). In the succeeding discussion, I will refer to Stage 1, 2, 3, 4 and 5 as steps in the process of development of a new pesticide. Promotion to each higher stage represents a formalized

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In brief, Stage 1 is the exploratory stage. Stage 2 identifies the key questions whereby limitations of a new discovery hopefully can be identified early. Stage 3 involves the commitment of extensive time and lots of money covering efficacy, toxicity, process, residues, metabolism, and ecological studies. Stage 4 involves (a) cooperative research outside of Dow to obtain the necessary confirmation of efficacy and safety and (b) the registration of the new product. Stage 5 is the initial sale.

The costs of Stage 1 are shown in Figure 9. A half million dollars may be involved by the end of Stage 1. The end point of Stage 2 may have cost a million dollars (Figure 10). In this case the product was flunked out on the basis of residue and metabolism work with accumulated costs of one million dollars. $1,900,000 could be invested by the end of Stage 3 and consume four years of time (Figure 11). In this case the production process was the deciding limitation. The product cost too much. A very expensive loser is one which flunks out in Stage 4 (Figure 12). In this case registration limitations were the limiting factor. Another example of a loser is shown in Figure 13, where 2.1 million dollars were invested at the time the project was terminated. Incidentally, that same money invested at 8% interest would result in a cash position of plus 3.4 million dollars, principal plus interest, as opposed to a loss of 2.1 million dollars (Figure 14). On the other hand a winning product for which there is still substantial hope involves a negative cash position of 4.6 million dollars (Figure 15) compared to a plus 6.1 million dollars (Figure 16) had that money been alternately invested at 8% where both principal and interest are shown above the line.

A winner is defined as a project with a market lasting at least 9 years at an annual sales level of 10-20 million dollars per year and a return on investment of 40% before tax. A good investment all by itself, but remember that the winners must support the losers. The total agricultural business is a mixture of winners and losers—and for some "real life" examples note (Figure 17). Project A was terminated after spending $600,000. Project B after spending $1,000,000. Project C, an expensive one, after spending $4,000,000. Project D after spending $1.5 million, and Project E after spending $0.5 million. Projects F, G and H are continuing and hopefully will result in winners.

The time to kill a loser is in early Stage 1, and hopefully no later than the end of Stage 2. The present costs as they relate to safety

FIGURE 12—Sunk Costs—Stage IV.

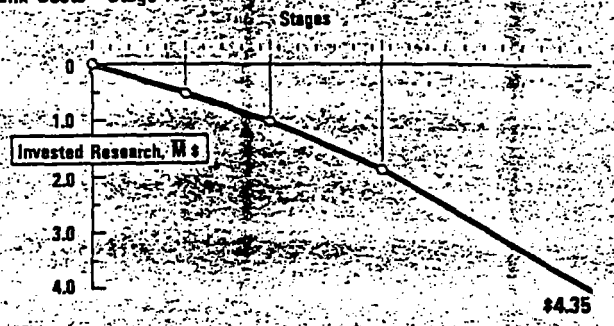


FIGURE 13—Cumulative Cash Position of a "Loser"—A.

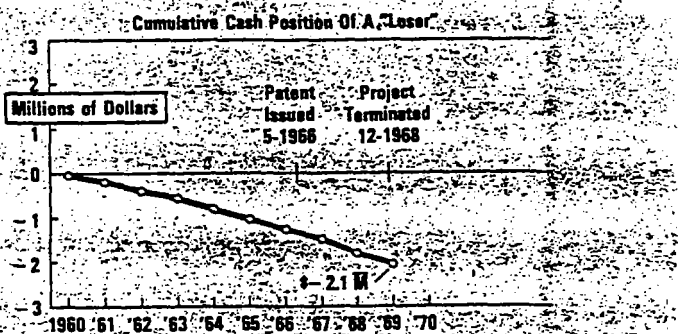


FIGURE 14—Cumulative Cash Position of a "Loser"—B.

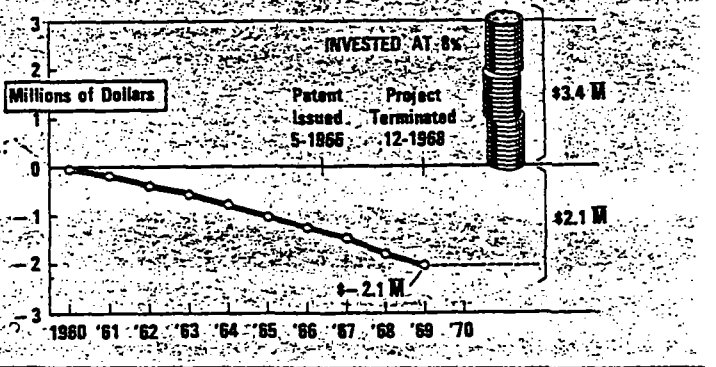
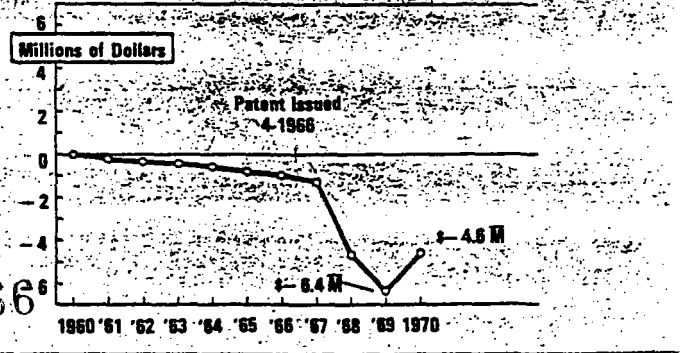
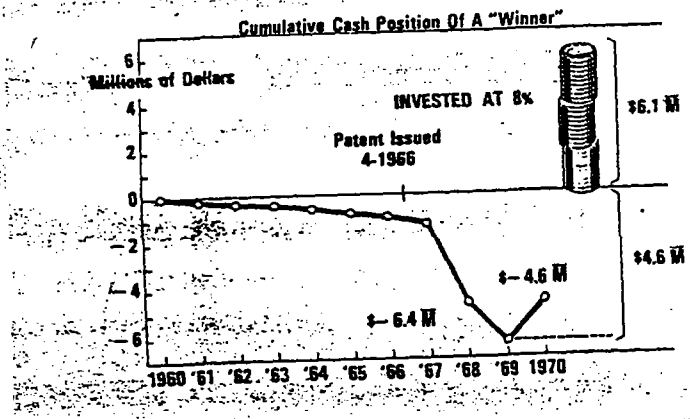


FIGURE 15—Cumulative Cash Position of a "Winner"—A.

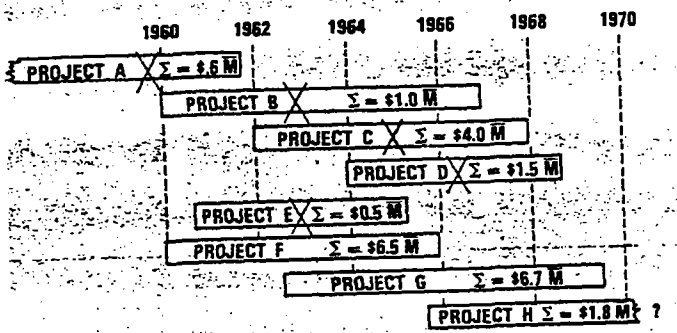


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GURE 16—Cumulative Cash Position Of A "Winner"—B.



GURE 17—Expenditure Pattern, Eight Typical Agricultural R & D Projects

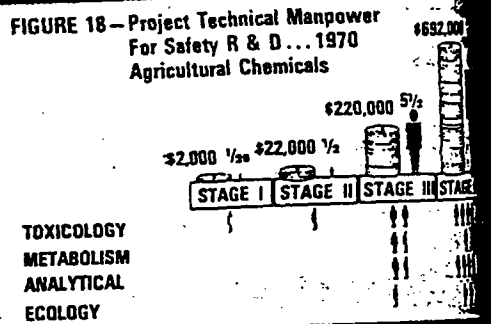


IGURE 19—Safety Evaluation For New Pesticide—Minimum Registration Requirements, Past, Present and Possible Future

	1950	1960	1970	1971-'80 (?)
OXICOLOGY	Acute, Lab. Animals 30-90 day, rat	Acute, Lab. Animals 90 day, rat 90 day, dog 2 year, rat 1 year, dog	Acute, Lab. Animals 90 day, rat 90 day, dog 2 year, rat 2 year, dog Reproduction, 3 gen., rat Teratogenesis, rodent Fish, shellfish, etc. Birds	Acute, Lab. Animals 90 day, rat 90 day, dog 2 year, rat 1 year, dog Reproduction, 3 gen., rat Teratogenesis, rodent Fish, shellfish, etc. Birds 2 yr., hamstar (carcinogenesis) Primate Human Mutagenesis
IETABOLISM		Animal (min.)	Rodent, and/or dog Plant	Rodent, and/or dog Plant Human
NALYTICAL	Food crops, 1 ppm*	Food crops, 0.1 ppm** Meat 0.1 ppm Milk 0.1 ppm	Food crops, 0.01-0.05 ppm** Meat 0.1 ppm Milk 0.005-0.05 ppm	Food crops, 0.01-0.05 ppm** Meat 0.1 ppm Milk 0.005-0.05 ppm
COLOGY			ENVIRONMENTAL Stability Movement Spectrum Accumulation	ENVIRONMENTAL Stability Movement Spectrum Accumulation

Pesticide ** Pesticide plus toxic metabolite(s)

FIGURE 18—Project Technical Manpower For Safety R & D...1970 Agricultural Chemicals



and ecological considerations in Research and Development are shown in Figure 18. Early toxicological work costs about $22,000 through Stage 2. Other safety costs (metabolism, analytical and effect on environment) increase toxicology expenses in Stage 3 totalling $220,000. Further expanded work continued through Stage 4 results in an accumulated expense of $692,000. But the most important consideration is *time.* The time value of money and the effective time of life of a patent are often underrated.

Future

Let's compare the toxicology requirements in 1950, 1960 and 1970 with the potential needs that *might* become requirements between '71 and '80 as presented in Figure 19. These would include additional testing for carcinogenesis, primate toxicology, human toxicology and mutagenesis. Perhaps human metabolism will also be required, plus intensified information on environmental effects. You will recall the $692,000 figure for the cost of toxicology, metabolism, analytical and ecology testing in 1970. The added requirements could involve another $400,000-$500,000, but cost is not as important as the added time. Once 5 million dollars has been invested a three year delay could cost an added 1 million dollars in terms of interest expense and losses of earnings while the project is held up. Developing pesticides is truly a high risk business. Changes and improvements will have to be made in the way we manage our destiny.

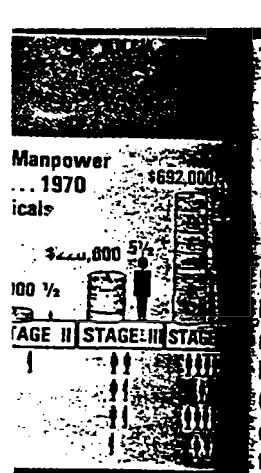
I would like to submit some suggestions for consideration. In the first place, the government should do a better job of defining test requirements, yet the test methods or protocols need flexibility, individual judgment, *and* provision for deliberate change. Change in requirements should not be capricious but should derive from advances in science and advances in new knowledge. Any change, however, should involve understanding and participation by industry, not by sudden unilater

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government decree. Changes should be made with good and sufficient reason based on fact.

Another suggestion is that a mechanism be developed for certification of toxicological laboratories. This means industrial laboratories, university laboratories, commercial laboratories and government laboratories. Greater public confidence could be developed for the results issuing from such laboratories if certification required that a qualifying board periodically rule on the adequacy of personnel, procedures, equipment and housing. The objective would be to improve and make more uniform the quality of the data used for support of products.

Assuming that laboratories could be certified, then only toxicological data from certified laboratories should be admissible for the support of product registration or for the support of residue tolerance. It is further suggested that the second supplier of a pesticide after the original petitioners patent expires, be permitted to purchase certified data. This could be purchased either from the original supplier or perhaps from a government agency. The main objective would be to burden the second supplier so that he does not have an unfair competitive advantage compared to the one who has borne the original cost of development.

With a provision of certified data being the only type of toxicological data admissible for support of tolerance, and with the requirement that the second supplier be required to pay his way, then it would be possible to lay the registration and the tolerance petitions open for inspection and to encourage publication. Openness of the data at this point would relieve many objections. Today neither the qualified investigator nor the public have access to the facts if they want them, hence the public is suspicious. Moreover, the experiment stations, expected to help support the use of a product, do not have access to certain registration information. Experiment stations and extension specialists need confidence in the validity of the back-up information. Moreover, the public official charged with registration and tolerance proceedings is under pressure because the present policy forbids him from making certain information available without consent of the petitioner. The whole situation promotes public distrust because supporting facts are not out in the open.

We should more aggressively extend the use of the experimental labels prior to full registration so that typical, though limited, sale can be achieved under qualified supervision. This would permit the development of meaningful use experience with limited exposure of the total population. The objective would be to discover unexpected phenomena difficult or impossible to expose under laboratory or limited field test conditions.

One of the weakest links in the chain is in diagnosis of pest problems and application of pesticides. I do not wish to discredit those competent professionals who do a good job, nevertheless, in the country at large the process of diagnosis and application involves people, usually unskilled, poorly paid, and nonprofessional. We have many elegant tools but poor craftsmen. To overcome this difficulty I would suggest that we classify pesticides in two categories —*for professional use only* and *for non-professional use.* Those for professonal use could be applied by licensed professionals only—skilled in diagnosis and application. Moreover, since there is a scarcity of this kind of skill, a training of a new kind of professional should be developed by our agricultural schools. This will take time, but it's needed and the professional licenses should be granted only to those qualified. The grower would then pay for results and the whole process of pest control, including weed control, should be less poundage oriented and more result oriented. This will require some drastic rethinking on the part of industry on how they can make their contribution and reap their share of the reward. I am convinced that the American consumer can well afford to pay a higher percentage of the shelf price of food or fiber for pest control if the job is professionally done with well designed products. In other words, there is economic room to maneuver if everyone is compelled to compete by equitable regulations.

As a final note—I would strongly advocate that we worry less about conflict of interest and see to it that government, industry and university personnel communicate more effectively.

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2. *Science,* 168 (864-866) 1970.
3. Brief of the USDA, In The United States Court of Appeals For The District of Columbia Circuit (No. 24,434), November 2, 1970.
4. Kearney, Phillip C. Report Presented before a joint meeting on Pesticides, United Kingdom, Canada and United States. Sponsored by the Council on Environmental Quality and President's Cabinet Committee on the Environmental Working Group on Pesticides, Washington, D.C., November 5, 1970.
5. *Bioscience, 20* (1004-1007) 1970.

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(212) 418-5200

WRITER'S DIRECT DIAL

August 10, 1989

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AUG 14 1989
CARTWRIGHT ET AL

Mary E. Alexander, Esq.
Cartwright, Slobodin, Bokelman,
Borowsky, Wartnick, Moore & Harris, Inc.
101 California Street, Suite 2600
San Francisco, California 9411

Re: Davis v. The Dow Chemical Company

Dear Mary:

In Dow's continuing effort to comply with the Court's directions, Dow has searched its files for records of shipments or sales between 1954 and 1973 of products containing 2,4,5-T to the United States Forest Service. Dow has recently discovered certain records which may be responsive to the Court's most recent order and encloses them without conceding their relevance or admissibility at trial both in terms of product identification and time frame.

Please feel free to contact me if you have any questions or comments.

Very truly yours,

Rivkin, Radler, Dunne & Bayh

Josh H. Kardisch

JHK:lc
Enc.

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THE DOW CHEMICAL COMPANY

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FOREST SERVICE
DESCANSO RANGER STATION

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INVOICE TO
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FOREST SUPERVISOR
3211 FIFTH AVE.
SAN DIEGO CAL 92103

INSTRUCTIONS
MARK ALL PKGS & PERS R/PO. NSR;

SALES & CO USE TAX	PART OF ↓ ORDER	TERMS: 30 DAYS NET OR	FRT.: COLLECT OR	TRIP LEASE	INVOICE DATE	SHIPPED DATE	C
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QUANTITY ORDERED AND DESCRIPTION (PRICE F.O.B. Shipping Point unless indicated below)	UNIT PRICE	INVOICING QUANTITY	SHIPPING WEIGHT	AMOUNT
S- 5 GL PAIL ESTERON 99 CONCENTRATE 1W1514/23682-04-195	2.67 GL	40	392	106.80
L- 5 GL PAIL ESTERON 245 CONCENTRATE 10805W/23756-04-135	8.50 GL	20	224	170.00
1ST ITEM---FSN6840-924-5361/CS-07 S-00281/AC/--------2ND ITEM. FSN 68 40-924-5363/CS-07S-00281-----DEL Y 08 6-3		1		
			TAX	
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				276.80

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INVOICE TOTAL

NO DISCOUNT ALLOWED ON PLATES BLUGS COLOR CHANGE CHARGES

0-38
581

Requisition No. 174-54-71

Appropriation and/or Project 115-121.03.54

PURCHASE ORDER

U. S. DEPARTMENT OF AGRICULTURE
FOREST SERVICE
3211 Fifth Avenue
San Diego, CA 92103

This number must appear on all PACKAGES and PAPERS relating to this order.

Order No. 777-02-71

Date May 24, 1971

To (Seller): The Dow Chemical Co.,
U.S. Government Marketing,
2030 ARC
Midland, Michigan 48640

Consignee and Destination (Ship To): U. S. Forest Service
Descanso Ranger Station
Descanso, CA 92016

Invitation No.	Contract No.	Time for Delivery	Discount Terms
	CS-07S-00281 (AO)	within one week	Net

F. O. B. Point	Ship Via	Gov't. B/L No.
Destination		

Item No.	Articles or Services	Quantity	Unit	Unit Price	Amount
37.	6840-924-5361 Herbicide, POEE, 2,4 D - 4# acid equivalent (Registration #464-301) 8-5 gallon steel pail	40	gal	2.67	106.80
54.	6840-924-5363 Herbicide, POEE, 2,4,5T, 6# acid equivalent (Registration #464-302) 4-5 gallon steel pail	20	gal.	8.50	170.00
				TOTAL	276.80

ACCOUNTING INSTRUCTIONS: Payment for above will be made upon receipt of invoice mailed to:

U.S. DEPARTMENT OF AGRICULTURE — FOREST SERVICE
Forest Supervisor
3211 Fifth Avenue
San Diego, CA 92103

Ordered by:

Signature

Name JOHN J. CASTLE
Title ADMINISTRATIVE OFFICER

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PURCHASE ORDER

U. S. DEPARTMENT OF AGRICULTURE
FOREST SERVICE
3211 Fifth Avenue
San Diego, California 92103

Requisition No.: 67-02-71

Appropriation and/or Project: 102-970-01-54-701 (502) 493.33 / 493.33

Order No.: 500-02-71

Date: 11/30/70

This number must appear on all PACKAGES and PAPERS relating to this order.

To: The Dow Chemical Co. 125-14137, 101-61-54
U.S. Government Marketing
2030 ARC Attn: Mr. Ryan
Midland, Michigan 48640

Consignee and Destination: U.S. Forest Service
District Ranger
Descanso, California 92016

Invitation No.	Contract No.	Time for Delivery	Discount Terms
n/a	CS-07S-21959	25 days	net - 30 days

F. O. B. Point	Ship Via	Gov't. B/L No.
destination	n/a	n/a

Item No.	Articles or Services	Quantity	Unit	Unit Price	Amount
1.	Herbicide, esteron 245, 2,4,5-T, in 5 gallon pails - (Butoxy Ethanol Ester or Propylene Glycol Butyl esters)(4# acid equivalent per gallon)	110	ea gal.	6.14	675.40
2.	Herbicide, esteron 99 concentrate, 2D4-D 4# acid equivalent per gallon	110	gal.	2.83	311.30
				TOTAL	$986.70

BILLING INSTRUCTIONS: Payment for above will be made upon receipt of invoice mailed to:
U.S. DEPARTMENT OF AGRICULTURE — Forest Service
Forest Supervisor
3211 Fifth Avenue
San Diego, California 92103

Ordered by
Signature
Name: JOHN J. CASTLE
Title: Administrative Officer

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AD-1

PURCHASE ORDER

U.S. DEPARTMENT OF AGRICULTURE
FOREST SERVICE
3211 Fifth Avenue
San Diego, California 92103

This number must appear on all PACKAGES and PAPERS relating to this order.

Requisition No.: 70-02-71
Accounting and Appropriation Data: 115-121-03-52 (63%) #[illegible]
103-570-47-52-701 (37%) #[illegible]

Order No.: 483-02-71
Date: 11/30/70

Seller:
The Dow Chemical Company
U.S. Government Marketing
2030 ARC Attn: Mr. Ryan
Midland, Michigan 48640

Consignee and Destination:
Ship To: District Ranger
U.S. Forest Service
1440 E. First St., Room 408
Santa Ana, California 92701

Quotation No.	Contract No.	Time for Delivery	Discount Terms
n/a	GS-075-21959	25 days	net - 30 days

F.O.B. Point	Ship Via	Gov't. B/L No.
destination	n/a	n/a

Item No.	Articles or Services	Quantity	Unit	Unit Price	Amount
1.	Herbicide, esteron 245, 2,4,5-T, in 5 gallon pails - (Butoxy Ethanol Ester or Propylene Glycol Butyl esters)(4# acid equivalent per gallon)	20	gal.	6.14	122.80
2.	Herbicide, esteron 99 concentrate, 204-D (4# acid equivalent per gallon)	50	gal.	2.83	141.50

SHIPPING INSTRUCTIONS: Payment for above will be made upon receipt of invoice mailed to:
Forest Supervisor
U.S. DEPARTMENT OF AGRICULTURE - FOREST SERVICE
3211 Fifth Avenue
San Diego, California 92103

Ordered by: Stevens
Signature: [signature]
Name: Teresa G. Dupray
Title: Purchasing Agent

TOTAL $264.30

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Environmental Protection Agency
Chamblee, Georgia 30341

TOXICOLOGY

Some Effects of 2,4-D and 2,4,5-T on Ehrlich Ascites Tumor Cells In Vivo and In Vitro

Ernest M. Walker, Jr., Ph.D., Richard H. Gadsden, Ph.D., Loretta M. Atkins, B.S., and Glen R. Gale, Ph.D.

2,4-D and 2,4,5-T shows appreciable inhibitory effects on the *in vivo* development of the Ehrlich ascites tumor in mice.

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INTRODUCTION

The application of 2,4-D, 2,4,5-T, and similar herbicides to plants impregnates plant cells with a persistent, spurious auxin which prevents the fluctuations of physiological auxins required for normal growth and differentiation. Hanson and Slife[1] have made detailed descriptions of the chemical and morphological changes in plant bodies and cells following exposure to 2,4-D and related chlorophenoxycarboxylic acid herbicides. Such changes include: little or no root elongation, abnormal growth in the basal stem, abnormal leaf development, and a number of other disruptions that may lead to alteration or death of the plant. The fact that the effects of 2,4-D, 2,4,5-T, and similar herbicides on plants apparently depend upon an auxin-like action led to the assumption that these agents would not be expected to alter or damage mammalian cells. Certain developments, which center largely about the potential toxicity and teratogenic activities of these herbicides in animals, particularly 2,4,5-T, have led to serious reconsideration of this assumption.

The Bionetics Research Laboratory of Litton Industries, under NIH contract, tested various pesticides and related compounds for teratogenic activity.[2] 2,4,5-T, administered in honey or DMSO, proved to be teratogenic in mice by causing increased numbers of abnormal fetuses and a statistically higher proportion of litters affected. The chief anomolies observed in affected offspring were cleft palates and cystic kidneys. Similar results were seen in Sprague-Dawley rats given various oral doses of 2,4,5-T on days 10-15 of gestation. The highest dosage of 46.4 mg/kg/day resulted in 60% fetal mortality and a high incidence of abnormalities in the survivors. These investigators reported that 2,4-D also produced an increase in the proportion of abnormal litters in mice.

An anonymous report summarizing some studies of the National Cancer Institute covers, in part, the potential teratogenic activities of 53 compounds, with the most conclusive results being found for 2,4-D and 2,4,5-T.[3] Doses of 113 mg/kg of a butyl ester of 2,4,5-T, administered from days 6-14 of gestation in mice produced a total of 54% malformations in the mice born from treated mothers, as compared to 1% in those born from control mothers. Malformed mice were seen in the litters of all six treated mothers. A dose of 98 mg/kg of the butyl ester of 2,4-D, given during the same interval of gestation, produced an increased incidence of malformations in the litters of four of the six treated mothers.

Several entries concerning the anti-tumor activities of 2,4-D (NSC 2925) and 2,4,5-T (NSC 430) are listed in the *Negative Results of the Cancer Chemotherapy Screening Data* of the United States Public Health Service. Entry No. 22683 reports that twice daily injections of 250 mg/kg of 2,4-D for seven days caused 43% inhibition of Sarcoma-180 in Swiss mice. In addition, the same entry reports that 11 daily injections of 75 mg/kg 2,4-D caused 63% and 42% inhibition in two different experiments using Adenocarcinoma 755 in CBF_1 mice. However, the entry reports that daily injections of 250 mg/kg 2,4-D had no effect on L-1210 leukemia in BDF_1 mice (mice were treated daily for 30 days or until death). Entry No. 37090 reports that twice daily injections of 88 mg/kg of 2,4,5-T for seven days caused 60% and 43% inhibition of Sarcoma-180 in Swiss mice in two separate experiments. The same entry reports that 2,4,5-T had

The following abbreviations are used: 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; MEM, Eagle's minimum essential medium with Hanks' balanced salt solution; DMSO, dimethyl sulfoxide; TPCV, total packed cell volume of Ehrlich ascites tumor; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; i.p., intraperitoneally.

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TABLE 1. Effect of 2,4-D on Development of the Ehrlich Ascites Tumor In Vivo

Dose mg/kg/day[a]	Number of injections[b]	Average weight change (gm) T/C[c]	Mortality C	Mortality T	Average TPCV[d] T/C, ml	% inhibition	S.D.[e] T/C	P
45	6	-0.4/1.8	0/8	0/8	1.061/1.549	32	0.330/0.355	<0.05
65	6	-1.7/1.6	0/10	1/8	0.924/1.744	47	0.431/0.320	<0.0005
75	6	0.2/3.2	0/9	2/9	0.728/1.572	54	0.460/0.265	<0.0005
77	5	-0.1/5.3	0/9	2/9	0.983/2.000	51	0.398/0.330	<0.0005

[a]Dimethyl sulfoxide was the vehicle
[b]Injected intraperitoneally, once per day
[c]Treated/Control
[d]Total packed cell volume
[e]Standard deviation of the treated/control groups

TABLE 2. Effect of 2,4,5-T on Development of the Ehrlich Ascites Tumor In Vivo

Dose mg/kg/day[a]	Number of injections[b]	Average weight change (gm) T/C[c]	Mortality C	Mortality T	Average TPCV[d] T/C, ml	% inhibition	S.D.[e] T/C	P
62	6	1.7/3.7	0/8	1/8	1.235/1.772	30	0.341/0.250	<0.005
78	6	-3.3/1.6	0/10	4/10	0.485/1.744	73	0.321/0.320	<0.0005
80	6	-1.5/5.8	0/9	3/9	0.728/2.000	64	0.266/0.330	<0.0005
85	5	-0.3/3.2	0/9	3/9	0.580/1.572	59	0.286/0.265	<0.0005

[a]Dimethyl sulfoxide was the vehicle
[b]Injected intraperitoneally, once per day
[c]Treated/Control
[d]Total packed cell volume
[e]Standard deviation of treated/control

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little effect on Adenocarcinoma 755 in CBF_1 mice (11 daily injections of 44 mg/kg) or L-1210 leukemia in BDF_1 mice (mice were treated daily for 30 days or until death). Consideration of the foregoing studies of 2,4-D and 2,4,5-T, especially the implications of their teratogenic potentials, prompted us to investigate the effects of 2,4-D and 2,4,5-T upon Ehrlich ascites tumor cells, including certain *in vivo* and *in vitro* parameters.

MATERIALS AND METHODS

2,4-D and 2,4,5-T were provided in a highly purified form (99.0%) by the Pesticides Research Laboratory, Perrine, Florida. Thymidine-methyl-^{3}H, uridine-5-^{3}H, L-leucine-^{14}C, and sodium formate-^{14}C were obtained from New England Nuclear Corporation. Ascites tumor cells were maintained in BALB/c mice (Flow Research Animals, Inc.).

The investigation included the following parameters which have been described in earlier reports as indicated: rates of synthesis of DNA, RNA, and protein[4,5]; *de novo* synthesis of RNA purines[6,7]; development of the Ehrlich ascites tumor *in vivo*.[8] In addition, the survival times of mice bearing ascites tumor cells and treated with each herbicide were assessed.

DMSO was used as the solvent system for all parameters in the investigation of 2,4-D and 2,4,5-T except the survival experiments in which 20% DMSO in 0.9% saline was used. The volume of DMSO solution injected was 0.1 ml per 40 grams body weight of each control or treated mouse. In the *in vitro* experiments, the final concentration of DMSO was 1% (v/v), and the same concentration of DMSO was contained in all control vessels.

RESULTS AND DISCUSSION

Effects of 2,4-D and 2,4,5-T On Development of the Ehrlich Ascites Tumor In Vivo

The inhibitory effects of 2,4-D and 2,4,5-T on development of the Ehrlich ascites tumor *in vivo* are shown in Tables 1 and 2, respectively. Both compounds were effective inhibitors of tumor development, with 2,4,5-T appearing to be slightly more effective than 2,4-D at equivalent dose levels. Increasing the dosage of 2,4,5-T from six injections of 62 mg/kg/day to six injections of 80-85 mg/kg/day doubled the extent of inhibition of tumor development. Similar increases in dosage of 2,4-D

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TABLE 3. Effects of 2,4-D and 2,4,5-T Upon the Average Survival Times of Mice Inoculated with Ehrlich Ascites Tumor Cells

Compound	Dosage mg/kg/day[a]	Number of Injections[b]	Number of deaths during Treatment Control	Treated	Ave. Time of Survival In Days Treated Control	% of Control Survival Time	S.D. in Days Treated[c] Control	P
2,4-D	75	5	0	0	18.4	126	2.7	<0.025
			8	8	14.6		3.9	
2,4,5-T	70	5	0	1	20.6	141	6.8	< 0.025
			8	8	14.6		3.9	

[a]20% DMSO in 0.9% saline was the vehicle
[b]The first i.p. injections of 2,4-D or 2,4,5-T in the above vehicle were given 24 hours after inoculation of each mouse with approximately 6×10^6 Ehrlich ascites tumor cells. Mice received one injection per day for five days.
[c]Standard deviation of the treated/control groups in days.

did not result in such marked increases in the degree of inhibition as was observed with 2,4,5-T.

Effects of 2,4-D and 2,4,5-T on Average Survival Times of Mice Bearing Ehrlich Ascites Tumor Cells

The average survival times of mice previously inoculated with Ehrlich ascites tumor cells and treated with each herbicide are shown in Table 3. Both compounds produced moderate but significant increases in the average survival times of tumor-bearing mice. 2,4,5-T caused a slightly greater increase in the average survival time (41%) than 2,4-D (26%), but the 2,4,5-T group had a higher standard deviation for survival of mice in the group (±6.8 days) than seen in the case of the 2,4-D group (±2.7 days).

Effects of 2,4-D and 2,4,5-T on RNA Purine Synthesis In Vivo

Data presented in Table 4 demonstrate the effects of 2,4-D on *de novo* RNA purine synthesis *in vivo*. The effects of 2,4-D on RNA guanine synthesis were not consistent. Dosages of 75 mg/kg and 100 mg/kg appeared to produce slight stimulatory effects upon RNA synthesis when injected i.p. 24 hours prior to the injection of sodium formate-^{14}C. Averages of 16% increase in RNA guanine and 8% increase in RNA adenine were found when 100 mg/kg 2,4-D was injected i.p. 48 hours prior to the injection of isotopically labeled formate.

Table 5 shows the effects of 2,4,5-T on *de novo* purine synthesis *in vivo*. 2,4,5-T appeared to produce a slight stimulatory effect upon RNA adenine synthesis when injected i.p. 24 hours before the injection of sodium formate-^{14}C by the same route. The effects on RNA guanine synthesis 24 hours after injection were not consistent. However, 2,4,5-T, 48 hours after i.p. injection of 100 mg/kg, produced an average of 28% inhibition of RNA adenine synthesis and an average of 30% inhibition of RNA guanine synthesis. Thus, the rates of synthesis of RNA adenine-^{14}C and guanine-^{14}C were inhibited to about the same extent 48 hours after injection, indicating that the inhibitory effects may be exerted on the common pathway of purine synthesis (prior to synthesis of inosinic acid).

Effects of 2,4-D and 2,4,5-T on the In Vitro Incorporation of Radioactive Precursors into DNA, RNA, and Protein of Ehrlich Ascites Cells

The effects of 10^{-4} M 2,4-D on the *in vitro* incorporation of radioactive precursors into DNA, RNA, and protein are shown in Table 6. Incubation of the cells for periods of an hour or less with 10^{-4} M 2,4-D caused a small and probably insignificant increase in the incorporation of thymidine-methyl-^{3}H into DNA. Similar periods of incubation produced no notable effects on the incorporation of uridine-5-^{3}H into RNA or on the incorporation of L-leucine-^{14}C into protein. Incubation of the cells with 10^{-4} M 2,4-D for periods of two hours resulted in slight stimulation, producing average increases of 17%, 37%, and 22% in the rates of incorporation of radioactive precursors into DNA, RNA, and protein, respectively.

The effects of 10^{-4} M 2,4,5-T on the *in vitro* incorporation of radioactive precursors into DNA, RNA, and protein are shown in Table 7. Incubation of ascites cells with the compound for intervals of an hour or less produced slight increases in the incorporations of thymidine-^{3}H into DNA and uridine-5-^{3}H into RNA. Increasing the incubation period to two hours produced no further increase in the incorporation of isotopic precursor into DNA and resulted in the loss of the stimulatory effects of RNA synthesis seen after shorter incubation periods. Incubation of ascites cells with 10^{-4} M 2,4,5-T for an hour or longer resulted in stimulation of the incorporation of L-leucine into protein. Increases of 23% and 28% were obtained after one and two-hour incubation periods, respectively.

In summary, 2,4-D and 2,4,5-T showed appreciable inhibitory effects on the *in vivo* development of the

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TABLE 4. Effect of 2,4-D on RNA Purine Synthesis in Ehrlich Ascites Tumor Cells In Vivo[a]

2,4-D mg/kg	Hours after Injection[b]	CPM/mg Ba↔ RNA Guanine	CPM/mg Ba↔ RNA Adenine	% Change Guanine	% Change Adenine	Guanine Adenine
0	24	4608	3064	---	---	1.50
25	24	3645	2558	-21	-17	1.42
50	24	3552	2725	-23	-11	1.30
60	24	4876	2739	+ 6	-11	1.74
75	24	5087	3464	+10	+13	1.47
100	24	4036	2804	-12	+ 9	1.44
0	48	3336	1854	---	---	1.80
100	48	4712	2246	+41	+21	2.10
0	48	3387	2369	---	---	1.43
100	48	3074	2221	- 9	- 6	1.38

[a]2,4-D in DMSO, at the doses indicated was injected i.p. to groups of 3-5 mice per group.
[b]Represents interval between i.p. injections of 2,4-D and formate-^{14}C. Animals were sacrificed three hours after the i.p. injection of formate-^{14}C. Cells were removed and processed as described in reference 7.

TABLE 5. Effect of 2,4,5-T on RNA Purine Synthesis in Ehrlich Ascites Tumor Cells In Vivo[a]

2,4,5-T mg/kg	Hours after Injection[b]	CPM/mg Ba↔ RNA Guanine	CPM/mg Ba↔ RNA Adenine	% Change Guanine	% Change Adenine	Guanine Adenine
0	24	3657	1256	---	---	2.91
25	24	3419	1147	- 7	- 9	2.98
50	24	4563	1336	+25	+ 6	3.42
60	24	3646	1277	0	+ 5	2.86
75	24	3868	1401	+ 6	+12	2.76
100	24	4212	1448	+15	+15	2.91
0	24	4377	2593	---	---	1.69
25	24	3597	3448	-18	+33	1.04
50	24	4019	2777	- 8	+ 7	1.45
60	24	5370	3813	+23	+47	1.41
75	24	4160	2636	- 5	+ 2	1.58
100	24	2992	2796	-32	+ 8	1.07
0	48	3336	1854	---	---	1.80
100	48	2207	1067	-34	-42	2.07
0	48	3387	2369	---	---	1.43
100	48	2513	2038	-26	-14	1.23

[a]2,4,5-T in DMSO, at the doses indicated was injected i.p. to groups of 3-5 mice per group.
[b]Represents interval between i.p. injections of 2,4,5-T and formate-^{14}C. Animals were sacrificed three hours after the i.p. injection of formate-^{14}C. Cells were removed and processed as described in reference 7.

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TABLE 6. Effects of 2,4-D on the In Vitro Incorporation of Radioactive Precursors into DNA, RNA, and Protein in Ehrlich Ascites Cells[a]

Synthesis of	*Sample*[b]	*Hours of Incubation*[c]	*Average CPM*[d]	*Average % of Control*
DNA	Control	0[e]	69138	
	2,4-D	0	70207	102
	Control	1	62294	
	2,4-D	1	65667	105
	Control	2	48211	
	2,4-D	2	56266	117
RNA	Control	0	75788	
	2,4-D	0	69457	92
	Control	1	50149	
	2,4-D	1	49799	99
	Control	2	31856	
	2,4-D	2	43528	137
Protein	Control	0	7644	
	2,4-D	0	7424	97
	Control	1	7250	
	2,4-D	1	7111	98
	Control	2	5567	
	2,4-D	2	6771	122

[a]The cells were incubated with the appropriate radioisotopes for periods of 20 minutes, at the end of which the reactions were terminated by the addition of an equal volume of 10% TCA (trichloroacetic acid). Radioactive precursors and final activities were: for DNA, thymidine-methyl-^{3}H (10 μCi/ml); for RNA, uridine-^{3}H (10 μCi/ml); and for protein, L-leucine-^{14}C, uniformly labeled (2 μCi/ml).

[b]Concentration of 2,4-D, 10^{-4} M in incubation medium.

[c]Time interval between addition of 2,4-D and addition of appropriate radioisotope to cells.

[d]Average of two similar experiments in which duplicate tubes were included for each sample (average of four tubes).

[e]Cells were added to incubation tubes containing 2,4-D and radioisotope.

TABLE 7. Effects of 2,4,5-T on the In Vitro Incorporation of Radioactive Precursors into DNA, RNA, and Protein in Ehrlich Ascites Cells[a]

Synthesis of	*Sample*[b]	*Hours of Incubation*[c]	*Average CPM*[d]	*Average % of Control*
DNA	Control	0[e]	37425	
	2,4,5-T	0	39599	106
	Control	1	28877	
	2,4,5-T	1	33568	116
	Control	2	24498	
	2,4,5-T	2	26944	110
RNA	Control	0	39846	
	2,4,5-T	0	44453	112
	Control	1	34953	
	2,4,5-T	1	33714	96
	Control	2	29962	
	2,4,5-T	2	28765	96
Protein	Control	0	13588	
	2,4,5-T	0	12149	89
	Control	1	7488	
	2,4,5-T	1	9181	123
	Control 2	2	5379	
	2,4,5-T	2	6875	128

[a]The cells were incubated with the appropriate radioisotopes for periods of 20 minutes, at the end of which the reactions were terminated by the addition of an equal volume of 10% TCA (trichloroacetic acid). Radioactive precursors and final activities were: for DNA, thymidine-methyl-^{3}H (10 μCi/ml); for RNA, uridine-^{3}H (10 μCi/ml); and for protein, L-leucine-^{14}C, uniformly labeled (2 μCi/ml).

[b]Concentration of 2,4,5-T, 10^{-4} M in incubation medium.

[c]Time interval between addition of 2,4,5-T and addition of appropriate radioisotope to cells.

[d]Average of two similar experiments in which duplicate tubes were included for each sample (average of four tubes).

[e]Cells were added to incubation tubes containing 2,4,5-T and radioisotope.

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Ehrlich ascites tumor in mice and caused mild to moderate effects on selected parameters of nucleic acid and protein syntheses as assessed by the preceding *in vivo* and *in vitro* studies. However, the extents of these effects may not be adequate to explain the relatively marked and significant degrees of inhibition of tumor development *in vivo*. These findings, therefore, tend to indicate a need for determining the effects of 2,4-D and 2,4,5-T on a number of additional biochemical and pharmacological parameters of cellular metabolism in mammalian cells.

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Neuropsychiatric Findings in P.B.B. Exposed Patients by Jeoffrey Stross, M.D., University of Michigan Medical Center, Ann Arbor, MI; Robert K. Nixon, M.D., Henry Ford Hospital, Detroit, MI; Marvin D. Anderson, M.D., Henry Ford Hospital, Detroit, MI.

Forty-four Michigan farm members underwent extensive clinical investigation for possible health effects secondary to significant P.B.B. exposure. Included in the study were psychiatric-psychologic evaluations and neurologic testing including standard electromyographic and nerve conduction determinations. Complaints of depression, memory-concentration defects and paresthesias were frequent. Results indicate the presence of a reactive rather than an organic depression in these patients. Neurologically, a significant incidence of peripheral neuropathies, chiefly sensory, were found by nerve conduction studies. There was no correlation between these findings and tissue levels of P.B.B. It is concluded that there are neurologic changes which may be due to tissue toxicity by P.B.B. The depression encountered in these patients was not of an endogenous organic character.

-24-

NEUROPATHOLOGY OF "SPINNING SYNDROME" INDUCED BY PRENATAL INTOXICATION WITH A PCB IN MICE by S.M. Chou, MD.,PhD., T Mirke, MD., W.M. Payne, MD., and G.J. Davis, PhD., West Va Univ Med Center, Morgantown, W.Va.

A striking motor disturbance, "spinning syndrome," developed with a high frequency in the weaning mice whose dams received oral 3,4,3',4'tetrachlorobiphenyl(4-CB) during the gestation. The syndrome is permanent and is characterized by swift circling movement (50 to 150 turns/min), restlessness and hyperkinesia. The spinners (24) of varied ages along with age-matched controls were subjected for histopathologic, histofluorescent and electron microscopic studies. The most reliable histopathologic marker for the spinners is the presence of cylindrical CNS peninsulas(CCPs) in the spinal nerve and cranial nerve roots. The CCPs consist of either CNS-type myelinated fibers or astroglial bundles and are enclosed in a basement membrane. A disturbance in synaptogenesis by 4-CB is thought attributable to the development of the CCPs. Interferrence with synaptogenesis may have also occured in the dopaminergic system. This likelihood is suggested by electron microscopic and histofluorescent catecholamine studies on the nucleus accumbens and the nigro striatal pathway and also by the responses on administration of dopaminergic agents, dopaminergic receptor stimulants and blockers.

-25-

LONG-TERM TOXICOLOGIC STUDIES OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN LABORATORY ANIMALS by R.J. Kociba, D.V.M.,Ph.D., D.G. Keyes, B.S., J.E. Beyer, B.S., R.M. Carreon, B.S. and P.J. Gehring, D.V.M., Ph.D., Dow Chemical USA, Midland, Michigan.

Rats were given diets supplying 0.1, 0.01, and 0.001 ug TCDD/kg/day, equivalent to 2200, 210 and 22 ppt of TCDD for 2 years. Ingestion of 0.1 ug/kg/day caused multiple indications of toxicity, including increased mortality, decreased weight gain, increased excretion of prophyrins and morphologic changes in multiple organ systems, especially the liver. This high dose caused an increased incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate/nasal turbinates or tongue, whereas a decreased incidence was noted in the pituitary, uterus, mammary gland, pancreas and adrenal. Rats given 0.01 ug/kg/day had a lesser degree of toxicity, limited primarily to increased urinary excretion of porphyrins plus some liver and lung lesions. Ingestion of 0.001 ug/kg/day caused none of the adverse effects seen with higher dose levels. Thus, continuous doses of TCDD sufficient to cause severe toxicity increased the incidence of some types of tumors, while decreasing other types. No increase in tumors occurred in rats given sufficient TCDD to induce slight or no toxicity.

-26-

LONG TERM EFFECTS OF TCDD AND HCDD IN MICE AND RATS by P. A. Holmes, J. H. Rust, W. R. Richter and A. M. Shefner, IIT Research Institute and University of Chicago, Chicago.

Tetrachlorodibenzo-p-dioxin (TCDD) and a mixture of isomers of hexachlorodibenzo-o-dioxin (HCDD) were given by gavage in corn-oil and acetone to mice and rats and skin-painted in acetone on mice for 2 years. HCDD in female rats slightly increased hepatic tumors but neoplastic nodules were increased strikingly. HCDD treated rats of both sexes had a marked multifocal pulmonary adenomatosis. In male mice, HCDD and TCDD by gavage increased hepatic tumors. In female mice both TCDD and HCDD markedly increased epithelial tumors.

In mice whose backs were painted with a solution of TCDD or HCDD, skin tumors increased in numbers and in aggression. DMBA pretreatment had no influence in either case. This study suggests that TCDD and HCDD are weak carcinogens given orally and are complete carcinogens when applied to the skin.

These studies were carried out under Contract NO1-CP-12338 with the National Cancer Institute and sub-contract 74-22-106002 with Tracor Jitco, Inc. (Contract NO1-CP-43350 with NCI).

-27-

STUDIES WITH CHLORINATED DIBENZO-P-DIOXINS, POLYBROMINATED BIPHENYLS AND POLYCHLORINATED BIPHENYLS IN A TWO-STAGE SYSTEM OF MOUSE SKIN TUMORIGENESIS: POTENT ANTI-CARCINOGENIC EFFECTS. David L. Berry, Ph.D., Thomas J. Slaga, Ph.D., Oak Ridge Nat'l Lab., Oak Ridge, TN., John DiGiovanni, Ph.D., and Mont R. Juchau, Ph.D., Univ. of Washington, Seattle, WA.

Topical pretreatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or Aroclor 1254 (PCB) inhibited the skin tumor-initiating ability of the polycyclic aromatic hydrocarbons (PAH) 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BaP) in female CD-1 mice. TCDD inhibited skin tumor-initiation by DMBA in a time dependent relationship. TCDD and Aroclor 1254 were very weak skin tumor initiators at doses of 2 ug and 100 ug respectively, topically followed by 32 weeks of promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA). The potency of the anti-carcinogenic effects resulting from pretreatment with TCDD and PCB correlate with the ability to induce epidermal monooxygenase enzymes that convert the PAH into reactive electrophilic intermediates and with the marked reduction in amounts of DMBA bound covalently in vivo to epidermal DNA and RNA but not protein. *Research sponsored jointly by NCI Grant CA20076 and Div. of Biol. & Environ. Res., U.S.DOE under contract W-7405-eng-26 with the U.C.C.

-28-

REPRODUCTIVE EFFECTS OF HALOGENATED AROMATIC HYDROCARBONS ON NONHUMAN PRIMATES by J.R. Allen, D.A. Barsotti, L.K. Lambrecht and J.P. Van Miller, Univ. of Wisconsin, Madison, WI.

Polychlorinated and polybrominated biphenyl mixtures and 2,3,7,8-tetrachlorodibenzo-o-dioxin have been fed at low levels to female rhesus monkeys over an extended period. Their general effects on reproduction are quite similar with modifications in serum estradiol and progesterone being associated with changes in the menstrual cycle. Difficulties with conception, early abortions and birth of undersized infants were observed. Slow growth, thymic involution, and increased susceptibility to infection were present in the postnatal period. Altered learning and behavioral patterns of the infants were also noted.

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Mutagenic Effects of TCDD on Bacterial Systems

By S. Hussain, L. Ehrenberg, G. Löfroth and T. Gejvall, Radiobiology Department, Wallenberg Laboratory, University of Stockholm, Sweden

TCDD, which occurs as a contaminant in the herbicide 2,4,5-T and possibly in other compounds derived from 2,4,5-trichlorophenol, is among the most toxic substances known. 2,4,5-T is widely used as a defoliant. The authors of this report claim that TCDD is mutagenic. Their experiments indicate that the mutagenic effect of TCDD is caused by intercalation with DNA.

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) occurs as an impurity in 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and possibly also in other compounds derived from 2,4,5-trichlorophenol (1). TCDD was early implicated as the cause of chloracne in workers subjected to occupational exposure in 2,4,5-T plants (2, 3). TCDD contamination as high as 30 ppm in 2,4,5-T has been reported (4), but 2,4,5-trichlorophenol that is currently produced is reported to contain less than 1 ppm (1). In addition, TCDD is formed when vegetation treated with 2,4,5-T is burned (5).

TCDD seems to have an exceptionally high toxicity. An LD_{50} (a dose lethal to 50 % of the population) of 0.6 μg/kg has been reported for female guinea pigs (6). A single oral dose of 10 μg/kg in rabbits is lethal and 1 μg/kg causes serious liver damage and chloracne (1), whereas rats seem to be less sensitive in view of the fact that ten consecutive oral doses of 8 μg/kg each, given daily during pregnancy, caused death only in one out of eight treated animals (7). In the same study on rats (7), foetal toxicity was observed at a dose level of ten consecutive doses of 0.125 μg/kg each.

Studies on genetic effects of TCDD have not been published except for a preliminary note on some data from the present report (8). The reason for the initiation of the experiments described here is that the three ring structure of TCDD resembles that of acridines and other compounds which induce mutations through intercalation

1. Reversion to streptomycin independency in *Escherichia coli* Sd-4. A test procedure which has been described previously was followed for the most part (9, 10). The required amount of TCDD (11), dissolved in 0.2 ml dimethyl sulphoxide (DMSO), was added to the bacteria in lag phase suspended in 0.8 ml of a 0.1-M sodium phosphate and potassium phosphate buffer of pH 7.0 containing 4.0 g/l sodium chloride and 0.2 g/l magnesium sulphate. The treatment was carried out at 37°C for one hour and was stopped by diluting ten times with buffer solution at 4°C.

The results are summarized in Table 1 and show that there is a high mutation frequency at a TCDD concentration of about 2 μg/ml and at a survival which excludes a preferential selection of spontaneously occurring mutants. Parallel tests with acridine orange showed that the mutation frequency could be raised to 50 per 10^8 at a concentration of 60—70 μg/ml and a survival of about 50 per cent.

TCDD concentration μg/ml	Survival %	Mutation frequency x 10^{-8}	Number of mutants counted
0	(100)	2.2	7
0.5	100	3	6
1*	90	2	3
1*	90		
2*	18	34	10
2*	11	256	46
4	3	(20)	1

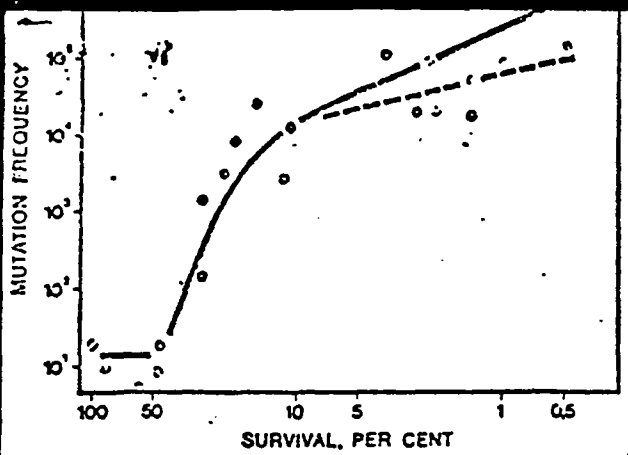
Table 1. Reversion to streptomycin independency in *E. coli* Sd-4 treated with TCDD.
*) Duplicate samples during treatment. A

2. Reversion to histidine prototrophy in *Salmonella typhimurium* strains deficient in uv excision repair. A treatment procedure similar to that used for *E. coli* was followed except that only 0.1 ml DMSO was added to 0.9 ml of the bacterial suspension. For the determination of survival, the treated bacteria were plated on nutrient agar. The minimal medium described by Hartman (12) and supplement according to Ames (13) was used for the determination of mutants. The mutant colonies were counted after four days.

i) *S. typhimurium* strain TA 1530. This strain has a base substitution in one codon for the first enzyme of the histidine biosynthesis. A one hour treatment with TCDD concentration of 1 and 10 μg/ml resulted in a survival of 90 and less than 1 per cent, resp. No mutations above the spontaneous level were found in this range.

ii) *S. typhimurium* strain TA 1532. This strain is assumed to have a base pair in excess of the aminotransferase locus. The effects of acridine mustard (ICR-170), dissolved in both DMSO and water, were investigated parallel to the tests with TCDD dissolved in DMSO (Fig. 1.). No increased mutation frequency was observed at concentrations of TCDD and acridine mustard which caused a survival in the range of 50—100 per cent, i.e. at concentration less than 2—3 μg/ml. At higher concentrations and lower survival the mutation frequency increased, reaching 10^3—10^5 per 10^8 surviving bacteria for TCDD around one per cent survival. Acridine mustard, dissolved both in DMSO and water, gave slightly lower

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pressed as number of mutants per 10^8 surviving cells, versus survival in *S. typhimurium* TA 1532.
° treatment with TCDD in DMSO
• treatment with acridine mustard in DMSO
• treatment with acridine mustard in water

3. Prophage induction. *E. coli* K-39 (λ) cells were harvested in log phase, washed, diluted to about 10^9 cells/ml and treated for 0.5 hour under conditions described above: 0.1 ml DMSO was added to 0.9 ml of the cell suspension. Following the ten-fold dilution with buffer solution at the end of the treatment, the bacteria were once more washed with buffer solution and then suspended in 1 ml nutrient broth and incubated for 2 hours at 37°C. Plating was done with *E. coli* K-49 as indicator on an appropriate medium (14). The plaques were counted after 18 hours. The applied DMSO inhibited the release of phage in comparison with the control test without DMSO, whereas TCDD at a non-toxic concentration of 0.5 μg/ml partly reversed this effect, producing an increase in the number of plaques from 1.9×10^5 per ml in the DMSO control to 5.3×10^5 per ml in the case of TCDD (Table 2).

Treatment of *S. typhimurium* TA 1530 with TCDD did not show any mutagenic effect, whereas high mutation frequencies were observed in the strain TA 1532 which is known to revert by frameshift mutation. This indicates that an acridine-like behaviour causes the genetic effects of TCDD.

The prophage induction was difficult to detect, but there seems to be a certain activation of phage in the presence of TCDD and DMSO which is not higher than the control value without DMSO. Although it is difficult to disentangle the effect of DMSO from that of TCDD, the data could be interpreted in terms of TCDD's weak prophage-inducing action, in view of the similar action of acridines (15).

The strong mutagenic action of TCDD demonstrated here (evidently a consequence of intercalation) and the efficient induction of microsomal hydroxylases (16) suggests lines of investigation which could explain the extraordinarily high toxicity of this compound.

Besides TCDD, other chlorinated dibenzo-*p*-dioxins, originating from chlorinated phenols, may become environmental pollutants (17). Occupational exposure to pentachlorophenol of unknown purity has been reported to cause chloracne-like symptoms (18). Some of the widely used commercial formulations of polychlorinated biphenyls (PCB's) contain impurities of chlorinated dibenzofurans (19) having a three ring structure resembling that of dibenzo-*p*-dioxin. Although no analyses with respect to impurities have been reported it is noteworthy that a widespread intoxication due to ingestion of a formulation of polychlorinated biphenyls was associated with chloracne-like symptoms (20).

Table 2

TCDD concentration μg/ml	Bacteria surviving treatment, No./ml x 10^{-6}	Plaques No./ml x 10^{-5}
0 (without DMSO)	4.4	7.3
0 (with DMSO)	4.2	1.9
0.5	4.1	5.4
1	2.0	1.8
1.5	2.2	2.6
2.5	1.7	1.6

Table 2. Prophage activation*) in *E. coli* K-35 (λ) treated with TCDD.
*) The term "prophage induction" is purposely avoided here.

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Toxicity of Chlorinated Hydrocarbons and Related Compounds

A Review Including Chlorinated Dibenzodioxins and Chlorinated Dibenzofurans

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Various chlorinated technical compounds, namely, 2,4,5-trichlorophenol, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and European chlorinated biphenyls (Phenoclor DP6 and Clophen A60), have been found to be contaminated with trace amounts of chlorinated dibenzofurans or chlorinated dibenzodioxins. Toxic fat which produces hydropericardium in chickens also contains chlorinated dibenzodioxins. These and other technical chlorinated compounds such as the technical pentachlorophenol have been implicated in causing chloracne, liver disease, teratogenicity, x-disease in cattle, and chick edema. The literature on the toxicity of the chlorinated technical compounds is reviewed. It is mentioned that 2,4,5-T and the chlorinated biphenyls also induce porphyria. Whether the various disease entities are caused by the contaminants, combinations of the chemical and its contaminants or by the chemicals themselves needs further evaluation.

RECENTLY several chlorinated compounds, such as the polychlorinated biphenyl and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), have received a great deal of attention. Polychlorinated biphenyls (PCB) are increasingly found in our environment and 2,4,5,-T has been used extensively as an herbicide. Occasionally these and other chlorinated technical compounds have caused diseases that bear similarity to each other.

Some of these chemicals have been found to be contaminated with chlorinated dibenzofurans or chlorinated dibenzodioxins. The various diseases produced by one or more of the different technical compounds are discussed in this paper. Attention is focused on the various contaminants and their possible relationship to different diseases in a variety of species. The need for further well-coordinated epidemiological and experimental animal studies is pointed out.

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Chloracne

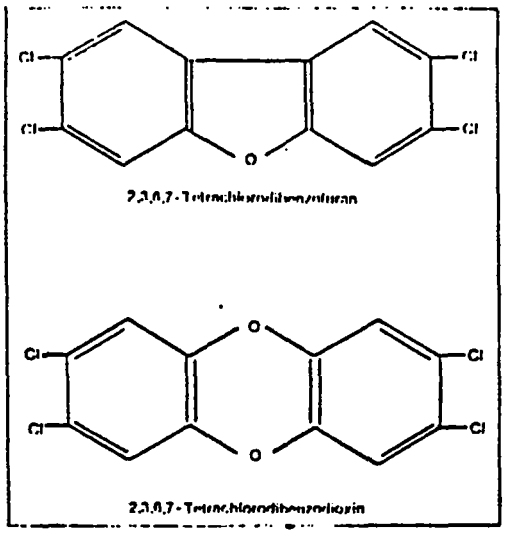
This disease is described by the formation of comedones with or without cysts and pustules. The follicular orifices are filled with sebaceous and keratinous material. Melanosis and a secondary inflammatory reaction may exist. Chloracne was described for the first time by Herxheimer[1] in 1899 who thought it was produced by free chlorine. Wauer[2] in 1918 and Teleky[3] in 1927 suggested the term "pernakrankheit." They felt that this skin disease was produced by certain chlorinated hydrocarbons. Jones and Alden[4] were among the first to report a case of chloracne in a 26-year-old male Negro who worked for three years distilling chlorinated biphenyls. This patient also complained of lassitude, loss of appetite, and loss of libido.

Chloracne is one of the most frequent forms of occupational dermatitis, and many cases have been reported in the United States as well as in Europe, particularly Germany and England.

Teleky[5] reported in 1949 that he personally had seen at least "150 to 280 workers" in four different factories with chloracne. He stated that liver disease occurs independently from chloracne, and usually manifests itself after an exposure time of four to six months, but may occur in as short a time as seven weeks. Occasionally loss of appetite, nausea, and edema of the face and hands are the first symptoms. Abdominal pain and vomiting follow, and then jaundice develops. At autopsy, acute yellow atrophy of the liver is found. He cites Flinn and Jarvik[6] who reported cases of acute yellow atrophy with fatal outcome following exposure to Halowaxes. Halowaxes, which are chlorinated naphthalenes, as well as the PCB (Aroclors) were increasingly used after 1930 to insulate cables. Teleky[5] cites many other case reports (Drinker et al,[7] Jones,[8] Greenburg et al,[9] Cotter,[10] McLechtchie and Robertson,[11] and Collier[12]). Von Oettin-

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Chemical structure of a chlorinated dibenzodioxin and a chlorinated dibenzofuran.

gen[13](pp308-310) describes the occurrence of chloracne in workers exposed to these chemicals. He cites several references which indicate that dermatitis is the result of the local effect or direct contact with the chemical, while the toxic hepatitis results from absorption.

Chloracne has been reported in workers exposed to chloronaphthalenes, chlorobiphenyls, chlorodiphenyloxides, certain petroleum products, and solid chlorophenols.[14] More recently Meigs et al[15] and Hofman and Meneghini[16] reported 14 and 13 cases of chloracne, respectively. Exposure to chlorinated biphenyls was established in most of these cases. Birmingham[17] observed chloracne in 15 employees who had painted flat sections of glass with enamel and then baked it. The enamel had Aroclor (a PCB) incorporated in it. Plewig[18] produced chloracne experimentally on the upper back of eight male adults by applying Halowax 1014, pentachloronaphthalene, and hexachloronaphthalene.

Bauer et al[19] reported chloracne in workers that handled technical 2,4,5-trichlorophenol. In some of these workers neuromuscular weakness, psychopathological changes, blepharoconjunctivitis, and liver involvement also occurred. Kimmig and Schulz[20] found that pure 2,4,5-trichlorophenol did not produce hyperkeratosis when applied to the rabbit ear. (The rabbit ear test is used as a screening test to determine whether a compound is likely to produce chloracne. The chemical, dissolved in propylene glycol, is painted on the rabbit ear. If the test is positive, erythema occurs within two days and in a few weeks hyperkeratosis develops.) The rabbit skin offers us an experimental method that will indicate the ability of substances to produce acneiform dermatitis in man.[21] Pure 2,4,5-trichlorophenol did not affect the rabbit ear, and neither did 1,2,4,5-tetrachlorobenzene, the starting material in the manufacture of 2,4,5-trichlorophenol. However, technical 2,4,5-trichlorophenol which was used in the production of 2,4,5-T did produce hyperkeratosis of the rabbit ear. It was concluded that by-products which had resulted during alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene were the causative agents.

Kimmig and Schulz[20] isolated several contaminants and found that some of them, namely tetrachlorodibenzofuran (Figure) at a concentration of 0.05% as well as tetrachlorodibenzodioxin (Figure) at a concentration of 0.005%, produced hyperkeratosis in the rabbit ear. A hepatotoxic effect was also observed with these compounds. They also found that pure pentachlorophenol did not produce hyperkeratosis of the rabbit ear. Chloracne was observed in workers of a West German plant who produced technical pentachlorophenol from hexachlorobenzene.[22] The technical material also induced hyperkeratosis in the rabbit ear. It is possible that technical pentachlorophenol contains toxic impurities, particularly since pentachlorophenol is produced by alkaline hydrolysis from hexachlorobenzene. Dibenzodioxin and dibenzofuran themselves do not cause liver necrosis or hyperkeratosis when applied to the rabbit ear; however, they become highly toxic when they contain three or more chlorine atoms.[20] Some of the workers with chloracne also develop eye irritation, hepatotoxicity, intolerance to alcohol, neuromuscular symptoms, porphyria cutanea tarda, and psychologic alterations.[23] According to Braun,[24] chloracne seems to result from direct contact with the chemicals producing it. Crow,[25] on the other hand, points out that chloracne can also be produced by systemic absorption.

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X-disease

This disease in cattle was described by Olafson[30] in 1947. A few years later evidence was published showing that highly chlorinated naphthalenes caused the disease.[27] Cattle with x-disease show a rapid decline in vitamin A plasma levels. Symptoms of poisoning include excessive lacrimation, diarrhea, polyuria, marked salivation, and discharge from the nostrils. A chronic cough, poor appetite, and numerous red maculae in the buccal mucosa develop, and hyperkeratosis of the skin follows. Microscopic examination of autopsy material shows central lobular degeneration of liver cells with bile duct proliferation and dilation of the glands in the wall of the gallbladder. Cystic dilation of the collecting tubules of the renal cortex, with a moderate degree of fibrosis and degenerated cells in the pancreas, was also observed.[28] In sheep, necrosis and cirrhosis of the liver, damage to the nephrons, and squamous metaplasia of the endometrium were the principle findings after ingestion of feed containing highly chlorinated naphthalenes.[29] Huber and Link[30] fed hexachloronaphthalenes to young swine and produced degenerative lesions of the liver and kidneys and hyperplasia of the vaginal epithelium with keratin formation. A depression of the vitamin A plasma level was also observed.

Drinker and co-workers[7] found that the liver of rats was affected when the animals were fed high doses of tetrachloronaphthalene, pentachloronaphthalene, and hexachloronaphthalene or chlorinated biphenyls. When Schoettle et al[31] fed hexachloronaphthalene to rats, they observed mild to moderate fatty degeneration of the liver with centrilobular vacuolation of hepatic cells. Degenerative changes were observed in the kidneys and the skin showed hyperkeratosis.

It can be concluded from these reports that highly chlorinated naphthalenes produce liver changes in several species. In cows, x-disease of the skin, together with a drop in the vitamin A plasma level, is one of the leading manifestations of the disease. It is possible that the vitamin A deficiency represents a manifestation of concomitant liver disease.

Sikes et al[32] found that x-disease could be produced in cattle with highly chlorinated naphthalenes, but also with petroleum products such as crank case oil. The toxic products were excreted in the milk and produced x-disease in the calves drinking the milk. They cited other authors who were able to produce the disease with a complex wood preservative and a lubricant.

Bell[33] tested the ability of various compounds of the chlorinated naphthalene group to produce x-disease. He discovered that dichlorinated and trichlorinated naphthalenes did not produce the disease while tetrachloronaphthalene had an effect, and the higher chlorinated naphthalene, pentachloronaphthalene, hexachloronaphthalene, heptachloronaphthalene, and octachloronaphthalene, caused severe disease. Octachloronaphthalene was less toxic than hexachloronaphthalene and heptachloronaphthalene.

Chick Edema

In 1957,[34,35] a disease occurred in a large number of chickens which, at first glance, seemed to represent an epidemic. It was soon discovered that the residues of certain distilled animal fats produced the condition when they were added to the chicken diet.[36,37] The disease was called chick edema because it manifests itself with hydropericardium and ascites in chickens. Ducks and turkeys experience a reduction in growth.

Allen and Lalich[38] produced hydropericardium and ascites in chickens when enough toxic fat was given to kill most of the birds in five weeks. ("Toxic fat" is a term used for fat found in chicken feed that induces chick edema.) When the concentration of toxic fat was reduced and fed to the chickens for 150 days, hydropericardium and ascites developed less frequently, but testicular hypoplasia became apparent. Simpson et al[39] described proliferation and hypertrophy of the vascular endothelium and possible necrosis of hepatic and bile duct tissue in chickens and turkeys. When monkeys (*Macaca mulatta*) were fed toxic fat, alopecia, subcutaneous edema, decreased total serum protein (with a reversal in the albumin-globulin ratio), and reduced hematopoiesis and spermatogenesis developed. Gastric ulcers occurred in 66% of the animals. Focal areas of necrosis and degeneration were observed in the liver; and the bile duct epithelium was found to be affected when it was examined under the electron microscope. Dilata-

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Table 1.—Disease Resulting From Exposure

Chloracne	Pathological Findings of the Liver	Birth Defects and Lethal Factor	X-Disease in Cattle
Certain petroleum products	Chlorinated naphthalenes	Certain technical 2,4,5-T	Highly chlorinated naphthalenes
Chloronaphthalenes (mainly penta, hexa, and hepta)	Chlorinated biphenyls	Purified material from toxic fat*	Petroleum products
Chlorobiphenyls	Technical 2,4,5-T and other chlorophenols	Polychlorinated biphenyls	
Chlorodiphenyloxides			
Chlorophenols (technical 2,4,5-trichlorophenol, pentachlorophenol)			
Technical 2,4,5-T			

* "Toxic fat" is a term used for fat found in chicken feed that induces chick edema.

tion of the heart and hypertrophy of the cardiac muscle were also present.[40]

Toxic fat is not the only product capable of producing the chick edema syndrome. Chlorinated biphenyl products, used as a plasticizer in a paint, have caused hydropericardium and ascites in chickens,[41] and two of seven Bengalese finches fed PCB developed hydropericardium.[42] The seven finches of a total of 56 birds died during the course of the experiment. A mixture of pentachloronaphthalene and hexachloronaphthalene, when fed to chickens, resulted in chick edema.[43] The toxic fat which contained the chick edema factor was studied intensively. Flick et al[44] separated a purified crystalline concentrate which produced chick edema and decreased the hatchability of injected eggs. Embryonic deformities were also produced. Flick et al[45] were not able to produce testicular hypoplasia in cockerels when they fed low doses of toxic fat (0.6% and 1.0%) to the cockerels for 12 weeks, as had been reported by Allen and Lalich.[38]

In 1967, Cantrell et al[46] reported that 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin was one of the toxic compounds producing chick edema. Tomita et al[47] had shown earlier that the heating of pentachlorophenol produced octachlorodibenzo-*p*-dioxin. Higginbotham et al[48] showed that the chick edema factor represented chlorinated dibenzo-*p*-dioxins. The two principal compounds isolated were 2,3,7-trichlorodibenzo-*p*-dioxin and 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin. The authors suggested, as a possible source of contamination, fats and fatty acids containing commercial chlorophenols. When crude fats and tallows are heated to produce fatty acids chlorophenol residues might be converted to a chick edema factor.

It was mentioned earlier in this article that the Aroclors can produce chick edema. The Aroclors (PCB) are used primarily as dielectric fluids for capacitors and transformers, as industrial fluids for hydraulic, for gas turbine and for vacuum pumps, and as heat transfer fluids. They are also used as plasticizers and are widely distributed in the environment.[49] Aroclor is a trade name under which these compounds are marketed in the United States. They can be found in synthetic resins, synthetic and natural rubbers, cellulose resins, paint varnish, wax, asphalt, and in allyl starch. They have been employed for dust prevention, moisture proofing, sealing, impregnation, and vapor suppression to prolong the residual life of insecticides. They increase the toxicity of dieldrin and DDT in insects.[50] Depending on the amount of chlorine they contain they are assigned certain numbers. Aroclor 1260 for instance, contains 60% chlorine, while Aroclor 1242 has an approximate chlorine content of 42%. Miller,[51] in 1944, tested a commercial chlorinated biphenyl with 42% chlorine and observed liver damage in rabbits, guinea pigs and rats. Skin changes were observed in the animals who received subcutaneous injections and application of the material to the skin. The skin lesions produced by subcutaneous injection were histologically similar to those of chloracne in man. Direct application to the skin produced inconsistent lesions compatible with low-grade irritation. Von Oettingen[13(pp306-307)] discusses some of the earlier work on the toxicity of the Aroclors in his book *The Halogenated Hydrocarbons, Toxicity and Potential Dangers*. Nishizumi[52] observed changes in the livers of mice and monkeys after feeding them chlorinated biphenyls. The author undertook this work because of an outbreak of poisoning that involved at

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to Technical Compounds
Chick Edema
Toxic fat*
Chlorinated biphenyls
Mixture of pentachloronaphthalene and hexachloronaphthalene

Table 2.—Toxic Contaminants Found in Some Technical Compounds*

2,4,5-trichlorophenol	Tetrachlorodibenzofuran Tetrachlorodibenzodioxin
2,4,5-T	Tetrachlorodibenzofuran Tetrachlorodibenzodioxin
Toxic fat	1,2,3,7,8,9 hexachlorodibenzo-p-dioxin 2,3,7 trichlorodibenzo-p-dioxin 2,3,7,8 tetrachlorodibenzo-p-dioxin
European chlorinated biphenyls (Phenoclor DP6 and Clophen A 60)	Tetrachlorodibenzofuran Pentachlorodibenzofuran Hexachloronaphthalene

* Listed in Table 1.

least 600 people in Western Japan who had ingested rice bran oil that had been contaminated with chlorobiphenyls, a Japanese PCB (Kanechlor 400). The chlorobiphenyls leaked into the rice bran oil through pinholes in a pipe used for heat exchange in the manufacturing process. An increase in pigmentation and abnormal grayish dark-brown pigment deposits in the skin of stillborn and newborn infants was observed.[53] Stillbirths were unrelated to PCB poisoning of the mother. Adults showed chloracne, anorexia, fatigue, edema of the eyelids, cheese-like discharge from the meibomian glands, and dark-brownish pigmented nails.[54]

Vos and Koeman[55] studied the toxicity of several polychlorinated biphenyls in chickens, quail and rats. The authors compared the toxicity of two European commercial polychlorinated biphenyls (Phenoclor DP6 and Clophen A60) and one (Aroclor 1260) which was produced in the United States. The European compounds showed higher toxicity and produced centrilobular liver necrosis. Chick edema was a common finding with European compounds and rare with Aroclor 1260. All three compounds produced porphyria. Chemical analysis of these three compounds revealed tetrachlorodibenzofuran, pentachlorodibenzofuran, and hexachloronaphthalene as a contaminant in the European samples but not in the sample from the United States.[56] Since hydropericardium occurred occasionally in chicks fed Aroclor 1260, the authors felt that this was indicative of small quantities of a toxic factor in this preparation.

Teratogenesis

Recently, Bionetics Research Laboratory, Bethesda, Md (unpublished data), as cited by Emerson et al[57] found a teratogenic effect of technical 2,4,5-T in rats. Apparently, the technical 2,4,5-T was contaminated with 27 ppm of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In the study published by Courtney et al,[58] it was stated that the technical 2,4,5-T which was found to be teratogenic in two strains of mice and one strain of rats was contaminated with 30 ppm 2,3,7,8-tetrachlorodibenzodioxin. Emerson et al[57] tested 2,4,5-T that only contained 1 ppm 2,3,7,8-tetrachlorodibenzo-*p*-dioxin at comparable dosage levels and did not elicit a teratogenic effect. This finding was substantiated by Sparschu et al[59] who studied teratogenic effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. A slight effect was observed at a dose of 0.125μg/kg/day when the material was given by gavage from day 6 through day 15 of gestation. At the level of 0.5μg/kg/day, the effect was quite pronounced. Courtney and Moore[60] were able to produce cleft palates and kidney malformations in three strains of mice with high doses (100 mg/kg subcutaneously daily from day 6 to 15 of pregnancy) of analytical grade 2,4,5,-T that contained less than 0.05 ppm 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Keplinger et al[61] reported a decreased survival of pups in rat reproduction studies when fed 100 ppm of Aroclor 1242 and 1254, and poor hatchability of eggs from chickens fed 10 or 100 ppm of Aroclor 1242 or 100 ppm of Arochlor 1254. It has been said[15] that a purified crystalline concentrate from toxic fat led to the development of embryonic deformities in chickens.

Comment

In some instances, the same chemical compounds are responsible for the occurrence of

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chloracne in people, liver disease in several species, x-disease in cattle, chick edema, and teratologic or other effects on the fetus. Not all of the disease entities have thus far been produced by all of the compounds (Table 1). Some of the compounds in question have been shown to be contaminated with chlorinated dibenzo-*p*-dioxins or chlorinated dibenzofurans (Table 2). The degree of contamination seems to vary with different compounds. Some companies manufacturing these compounds have instituted clean-up procedures which remove the greatest part of the contaminants. Improved hygiene among the workers and improved ventilation of the working area have, in many instances, reduced the incidence of chloracne. Whether all disease entities described are caused by the chlorinated dibenzodioxins or the chlorinated dibenzofurans is not yet known. Some of these chemicals are highly toxic, as illustrated by the fact that two applications of 10μg of 2,3,6,7,-tetrachlorodibenzo-*p*-dioxin applied to human skin[62] produced chloracne-like symptoms. Single oral doses of 20μg to 50μg/kg/body weight resulted in fatal liver necrosis in rabbits.[62] There is no readily available information on the metabolism of these compounds in animals and their breakdown in the environment.[63] It is probable that they are stable. Crosby et al[61] found that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and its homologues decomposed rapidly in alcohol solution under artificial light and natural light. However, photodecomposition was negligible in aqueous suspensions and on wet or dry soil. It is unknown whether the disease entities discussed are the result of one or several contaminants or the combined effect of the chemical compound and its contaminant.

Another unsolved problem is the observation that some of these compounds such as technical 2,4,5-T induce porphyria.[23] The chlorinated biphenyls also cause porphyria.[55] Whether porphyria is the result of the chemicals themselves or of the contaminants, or both, is not known. Crow[65] suggests that the porphyria produced by technical 2,4,5-T may have been caused by contamination of the chemical with crude chlorobenzenes, which were used to produce the chlorophenols from which the 2,4,5-T was made. Better awareness of the possible contamination of the technical chemicals with various highly toxic substances, well coordinated epidemiological studies, and additional experimental studies in animals are needed to clarify these problems.

Chloracne has been produced experimentally in men by Shelley and Kligman (*Arch Derm* 75:689, 1957). The authors cite additional references on experimental dermal exposure of animals and men to chlorinated naphthalenes.

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SUMMARY TERATOLOGY

THE NO-EFFECT DOSE LEVEL OF 2,4-D AND 2,4,5-T IN A VARIETY OF SPECIES IS IN THE RANGE OF 25 - 50 MG/KG/DAY THROUGHOUT ORGANOGENESIS.

2,3,7,8-TETRA~ AND HEXACHLORODIBENZO-P-DIOXIN ARE HIGHLY TOXIC TO THE DEVELOPING EMBRYO AND FETUS OF THE RAT; 1,2,3,4-TETRA~ , 2,7-DI~ AND OCTACHLORODIBENZO-P-DIOXIN ARE NOT.

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TERATOLOGY - 2,4-D

AUTHORS	SPECIES	FORM OF 2,4-D	ROUTE	DOSE LEVELS MG/KG/DAY
COLLINS & WILLIAMS, 1971 (C.6.8)	HAMSTER	ACID	ORAL	20 (40), 60, 100
KHERA & MCKINLEY, 1972 (C.6.6)	RAT	ACID, SALT	ORAL	25 (50), 100, 150
SCHWETZ ET AL, 1971 (PP 8F0670)	RAT	ACID, ESTER	ORAL	12.5 (25), 50, 75, 87

(○) = NO-EFFECT DOSE LEVEL FOR EMBRYO AND FETAL TOXICITY.

TERATOLOGY -2,4,5-T

AUTHORS	SPECIES	FORM OF 2,4,5-T	ROUTE	DOSE LEVELS MG/KG/DAY
COLLINS & WILLIAMS, 1971 (C.6.8) *	HAMSTER	ACID	ORAL	20, (40), 80, 100
COURTNEY ET AL., 1970 (C.6.9) *	RAT, MOUSE	30 PPM 2,3,7,8-TCDD		
COURTNEY & MOORE, 1971 (C.6.11) * (C.6.12) *	MOUSE	ACID	SC	(50), 100, 125, 150
	RAT	ACID	SC	10, 22, (46), 80, 250
KHERA & MCKINLEY, 1972 (C.6.6) *	RAT	ACID, SALT, ESTERS	ORAL	25, (50), 100, 150
NEUBERT & DILLMAN, 1972 (C.6.13) *	MOUSE	ACID	ORAL	8, 15, (30), 45, 60, 90, 12
	MOUSE	BUTYL ESTER	ORAL	12, 74, (<12)
ROLL, 1971 (C.6.14) *	MOUSE	ACID	ORAL	(20), 35, 60, 90, 130
EMERSON ET AL., 1971 (C.6.3) *	RABBIT	ACID	ORAL	10, 20, (40)
	RAT	ACID	ORAL	1, 3, 6, 12, (24)
SPARSCHU ET AL., 1971 (C.6.4) *	RAT	ACID	ORAL	50, 100, (<50)
BINNS & BALLS, 1971 (C.6.2) *	SHEEP	ACID ESTER	ORAL	100, (>100)

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○ = NO-EFFECT DOSE LEVEL FOR EMBRYO AND FETAL TOXICITY.

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*See PP 1F1102, May 1973

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TERATOLOGY - DIBENZO-P-DIOXINS

AUTHORS	-DIOXIN	SPECIES	ROUTE	DOSE LEVELS µG/KG/DAY
SPARSCHU ET AL, 1971 (C.6.5) *	2,3,7,8-TETRA~	RAT	ORAL	(0.03),0.125,0.5,(8)
KHERA & RUDDICK, 1971 (C.6.7) *	2,3,7,8-TETRA~	RAT	ORAL	(0.125),0.25,0.5,(2,4,8,16)
	1,2,3,4-TETRA~	RAT	ORAL	50,100,200,400,(800)
	2,7-DI~	RAT	ORAL	250,500,1000,(200
SCHWETZ ET AL, 1972 (C.7.5) *	2,7-DI~	RAT	ORAL	(100,000)
	HEXA~	RAT	ORAL	(0.1),1,10,100
	OCTA~	RAT	ORAL	(100,000),500,000
COURTNEY & MOORE, 1971 (C.6.11) * (C.6.12) *	2,3,7,8-TETRA~	RAT	SC	0.5 (<0.5)
		MOUSE	SC	1,3 (<1)
NEUBERT & DILLMAN, 1972 (C.6.13) *	2,3,7,8-TETRA~	MOUSE	ORAL	0.3,3,4.5,9 (<0.3)

*See PP 1F1102, May 1973

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THE DOW CHEMICAL COMPANY

Summary of Teratology Studies with 2,4-D

compiled by Marguerite L. Leng
The Dow Chemical Company, Midland, Michigan

October 18, 1972

Teratogenic studies have been conducted with 2,4-D and a number of its derivatives in rats (Schwetz et al. 1971, Khera and McKinley, 1972), in hamsters (Collins and Williams, 1971) and in sheep (Binns and Johnson, 1970). In each case, a no-effect level of at least 25 to 50 mg/kg/day was found.*

In the study conducted by Dow (Schwetz, 1971) 2,4-D and its propylene glycol butyl ether (PGBE) and isooctyl esters were given orally to pregnant rats at doses of 12.5, 25, 50, 75, and 87.5 mg acid equivalent per kg of body weight each day on days 6 through 15 of gestation. The highest dose (87.5 mg/kg/day) was chosen because levels higher than this caused signs of toxicity in the pregnant rats. Fetuses were taken by Caesarian section on day 20 of gestation. (The day on which sperm were seen in a vaginal smear was considered day 0 of pregnancy.) Signs of fetotoxicity and embryotoxicity were noted in fetuses of mothers given the highest dose levels daily for half her pregnancy, and included decreased fetal body weight, subcutaneous edema, delayed ossification of bone, lumbar ribs, and wavy ribs. These effects were not considered teratogenic because they would not seriously interfere with development or survival. No effect was noted at 25 mg/kg/day and minor effects were noted at 50 mg/kg/day.

In the study conducted by the Canada Food and Drug Directorate (Khera, 1972) 2,4-D and its butyl, isooctyl, butoxyethanol, and dimethylamine derivatives were given orally to pregnant rats at doses from 25 to 300 mg/kg/day, with most tests at 50, 100, or 150 mg/kg/day on days 6 through 15 of gestation, and the animals were killed on day 22 of pregnancy. (Sperma-

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*See also attached tabular summary of teratology studies with 2,4-D, 2,4,5-T and various dibenzo-p-dioxins (Schwetz 10/6/72)

tozoa in the vagina on the morning following overnight mating was considered day 1 of gestation.) They reported that effects noted at 50 mg/kg/day were not significantly different from those in untreated animals. Doses of 100 or 150 mg/kg/day of 2,4-D induced fetal deaths and caused an increased incidence of skeletal anomalies. Normally occurring anomalies included wavy ribs, extra ribs, delayed ossification of skull bones and several changes in the sternum. They also observed a low incidence of anomalies at the high dose levels which were not present in control animals. These included fused ribs and bent or distorted leg bones. Some animals were allowed to deliver normally and the weight gain and viability of the offspring were within normal limits.

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In the study conducted by the U.S. Food and Drug Administration (Collins, 1971), 2,4-D from three different manufacturers was given by oral intubation to hamsters at doses from 20 to 100 mg/kg/day on days 6 through 10 of gestation and the animals were sacrificed on day 14 of gestation. The incidence of fetal anomalies was low and consisted of delayed head ossification or ear abnormality in only two fetuses from three groups given 100 mg/kg/day, and fused ribs in a total of nine fetuses from treated groups compared to three in the control group. The authors concluded that the % abnormalities, even at 60 and 100 mg/kg/day, were not significantly different from the control percentage.

In the study conducted by the U.S. Department of Agriculture (Binns, 1970), pregnant ewes were given 2g of 2,4-D mixed with alfalfa meal each day for 30, 60, or 90 days after breeding. (This is equivalent to approximately 40 mg/kg/day in sheep). No signs of congenital malformations in the lambs, or clinical poisoning or histopathological lesions in the dams were noted.

Schwetz, B. A., G. L. Sparschu, and P. J. Gehring (1971). The Effect of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Esters of 2,4-D on Rat Embryonal, Foetal, and Neonatal Growth and Development. Food Cosmetics Toxicology 9, 801-817. (C.5.3)

Khera, K. S., and W. P. McKinley (1972). Pre- and Postnatal Studies on 2,4,5-Trichlorophenoxyacetic Acid, 2,4-Dichlorophenoxyacetic Acid and Their Derivatives in Rats. Toxicology and Applied Pharmacology 22, 14-28. (C.5.4)

Collins, T.F.X., and C. H. Williams (1971). Teratogenic Studies with 2,4,5-T and 2,4-D in the Hamster. Bull. Environ. Contam. & Toxicology 6(6), 559-567. (C.5.5)

Binns, W., and A. E. Johnson (1970). Chronic and Teratogenic Effects of 2,4-D (2,4-Dichlorophenoxyacetic Acid) and Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) to Sheep. Proc. N. Cent. Weed Contr. Conf. 25, 100. (C.5.6)

Addendum, August 1973.

Also attached are excerpts from two papers presented at the Eighth Inter American Conference on Toxicology and Occupational Medicine in Miami, July 1973.

(a) Clegg, D. J. and K. S. Khera. The Teratogenicity of Pesticides, Their Metabolites and Contaminants. (Phenoxy Herbicides p. 270-273)

(b) Durham, W. F. and C. H. Williams. Mutagenic, Teratogenic and Carcinogenic Properties of Pesticides. (2,4,5-T and 2,4-D p. 316-317)

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Effect of Tetrachlorodibenzo-p-Dioxin on Growth Rate and the Synthesis of Lipids and Proteins in Rats

by H. M. Cunningham and D. T. Williams
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DOW373315

Chick edema disease was first observed in 1957 when thousands of broilers were lost (1) but it was not until 1966 that chlorodibenzo-p-dioxins were found to be the causative agent (2). One of the most toxic dioxins was 2,3,7,8-tetrachlorodibenzo-p-dioxin (3). The most prominent toxic effects in chickens are hydropericardium and hydroperitoneum (4,5,6) while in mammals the liver appears to be the main point of attack showing enlargement and fatty infiltration (6, 7). In one report, enlarged livers in rats were not accompanied by an increase in liver fat (8). The present experiments were conducted to determine if dioxins affect the ability of the liver to synthesize lipids and protein, and the dosage and time required to produce maximum effects.

MATERIALS AND METHODS

In each experiment 12 to 16 weanling male Wistar rats were allotted at random into two to four treatment groups. 2,3,7,8-tetrachlorodibenzo-p-dioxin (99% pure, courtesy of the Dow Chemical Company) was dissolved in corn oil and given by oral intubation at levels of 0 to 10 µg/kg of body weight. Both treated and control rats received corn oil at a level of 10 ml/kg. One to seven days later each rat received an intraperitoneal injection of 1.0 µc of 1-^{14}C-L-leucine and 10.0 µc of ^{3}H-sodium acetate. Exactly 1 hr later the rat was killed by a blow on the head, the liver was removed and 1-g samples were homogenized with 4.0 ml of 0.9% NaCl. The lipids were extracted by a procedure similar to that of Folch et al. (9) and the radioactivity was counted with a liquid scintillation counter (10). In some experiments the lipids were partitioned by thin-layer chromatography (10). The proteins were separated from the aqueous phase of the Folch extraction by centrifuging, washing twice with 5% trichloroacetic acid and twice with 2:1 methanol: ether (v/v). They were dried under nitrogen and 20 mg aliquots were solubilized in scintillation vials with 1 ml of Soluene (Packard Instrument Company) at 55°C in a shaking water bath. In some experiments lipids were also extracted from adipose tissue and proteins isolated from heart and skeletal muscle.

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RESULTS

Experiment 1. A comparison was made between levels of 0, 1.0 and 10.0 µg of dioxin per kg of body weight on the incorporation of labelled acetate and leucine into liver lipids and proteins. The dioxin significantly reduced body weight gain ($P<0.05$) and the radioactivity of the free fatty acids in the liver ($P<0.01$) along with reductions in other liver lipid fractions (Table 1). The specific activity of liver protein, on the other hand, increased ($P<0.05$) when dioxin was given. The 10.0 µg/kg dose level appeared to have a greater effect on most measurements than the 1.0 µg/kg level but the differences between them were not large.

TABLE 1

Effects of Dioxin on Liver Lipid and Protein Synthesis 3 days after dosage

	Oral dioxin dosage (µg/kg)			
	0	1.0	10.0	S.E.
Number of rats	4	4	4	
Initial weight, g	49.5	51.2	51.0	
Weight gain per day, g	3.1	0.4	0.2	2.1*
Liver lipids				
(DPM ^{3}H/mg liver)				
total lipids	23.66	15.46	11.46	4.25
triglycerides	3.47	2.09	2.17	0.53
free fatty acids	0.31	0.13	0.08	0.05**
diglycerides	13.95	10.68	6.63	2.67
phospholipids	5.91	2.58	2.66	1.26
Liver protein				
(DPM ^{14}C/mg protein)	234	330	330	28

*P < 0.05
**P < 0.01

Experiment 2. The 10.0 µg level of dioxin was again compared with a zero control over a 3-day period. It again had a similar effect on growth rate and the incorporation of labelled acetate and leucine into lipids and proteins but with more rats per group more of the differences were significant at the 1% level of probability (Table 2). The ^{14}C-leucine incorporation by the heart was not affected by dioxin but there was a significant reduction ($P<0.05$) in the incorporation of ^{3}H-acetate by epididymal adipose tissue.

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TABLE 2

Effects of Dioxin on Lipid and Protein Synthesis in Liver Compared with that in Heart and Adipose Tissue 3 days after Dosage

	Oral dioxin dosage (ug/kg) 0	10.0	S.E.
Number of rats	8	8	
Initial weight, g	76.1	76.2	
Weight gain per day, g	2.25	0.36	0.35**
Liver lipids			
(DPM ^{3}H/mg liver)			
total lipids	15.62	6.92	1.86**
triglycerides	2.69	1.31	0.39*
free fatty acids	0.08	0.07	0.07
diglycerides	4.02	1.62	0.95
phospholipids	5.55	1.98	0.64**
Epididymal adipose tissue			
(DPM ^{3}H/mg lipid)	215.8	71.7	37.4*
Liver protein			
(DPM ^{14}C/mg protein)	166	223	6.9**
Heart protein			
(DPM ^{14}C/mg protein)	38	33	7.0

*P < .05
**P < 0.01

Experiment 3. The 10.0 µg level of dioxin was used to determine the number of days after dosing at which it would have the greatest effect on liver lipid synthesis (Table 3). There was a slight reduction in ^{3}H-acetate uptake by liver lipids (DPM/g of liver) at 2 days and a significant decline (P<0.05) at 7 days.

TABLE 3

Effect of Dioxin on Liver Lipid Synthesis at Varying Intervals After Dosage

	Days after giving 10 µg dioxin/kg Controls	1	2	3	7
Number of rats	4	4	4	4	4
Initial weight, g	61.5	64.0	65.7	62.0	63.5
Weight gain per day, g	3.4	2.5	1.9	2.8	3.1
Total liver lipids (DPM/mg liver)	16.38	16.79	13.78	14.35	9.00*

*P < 0.05

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Experiment 4. Graded levels of dioxin were used in this experiment to determine the smallest dosage that would have a noticeable effect on protein or fat synthesis 7 days later (Table 4). There was a progressive increase ($P<0.05$) in liver weight as the level of dioxin intake increased with a maximum increase of 21% at the 10 µg/kg level and a minimum of 9% at 0.1 µg/kg. There also was a trend towards reduced ^{3}H-acetate incorporation into liver and adipose tissue lipids with dioxin treatment but with only 4 rats per treatment the differences did not reach statistical significance. Part of the reduction in the incorporation of ^{3}H-acetate per g of liver could be accounted for by an increase in the size of the liver.

TABLE 4

Effects of Varying Levels of Dioxin on Lipid and Protein Synthesis 7 days after dosage

	Oral dioxin dosage (µg/kg)				
	0	0.1	1.0	10.0	S.E.
Number of rats	4	4	4	4	
Initial weight, g	63.2	64.2	69.2	65.7	
Weight gain per day, g	4.6	5.0	5.1	5.1	0.43
Liver weight, g	4.3	4.7	4.8	5.2	0.21*
Liver lipids, total (DPM ^{3}H/mg liver)	14.93	11.34	9.12	9.51	1.88
Epididymal adipose tissue (DPM ^{3}H/mg lipid)	322.3	125.2	56.3	138.0	86.0
Liver protein (DPM ^{14}C/mg protein)	186	168	153	143	17.0

Experiment 5. This experiment compared protein and fat synthesis at 3 and 7 days after dioxin dosage (Table 5). The dioxins had little effect on rate of body weight gain but liver weights were noticeably increased 3 days after treatment ($P<0.05$) and slightly increased after 7 days. The increase in liver weight was accompanied by a large increase in liver lipids which also was more apparent 3 days after dioxin treatment than after 7 days. The total incorporation of ^{3}H-acetate into liver lipids (DPM x 10^{3}/liver) was unaffected by dioxin but the increase in unlabelled liver lipids diluted the labelled acetate so that the specific activity of the liver lipids (DPM/mg lipids) was significantly ($P<0.05$) reduced.

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Adipose tissue in the flank region of rats incorporated considerably less ^{3}H-acetate into lipids than epididymal tissue in earlier experiments but dioxin appeared to have a greater inhibitory effect on incorporation at 7 days than at 3 days after treatment, although neither of the differences reached statistical significance.

The protein concentration in the liver (mg/g) was slightly reduced 3 days after dioxin was given, apparently due to the large dilution of the liver with lipids. At 7 days the protein concentration in the liver was unaffected by dioxin. The incorporation of ^{14}C-leucine into liver protein was significantly ($P<0.05$) increased 3 days after dioxin treatment and only slightly increased after 7 days. The specific activity of skeletal muscle was not affected by dioxin treatment.

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DISCUSSION AND CONCLUSIONS

Most of the animal experiments reported to date in the literature have used "toxic fats" or fractions of these fats containing mixtures of different chlorodibenzo-p-dioxins. The tetrachlorodibenzo-p-dioxin used in the present experiments was 99% pure and the highest dosage used (10 µg/kg) was close to lethal for when this amount was given orally to rats each day in a preliminary experiment they all died within 2 to 4 days. The lowest level in a single dosage that caused an increase in the liver weights of rats was 0.1 µg/kg. The dioxin had no effect on the rate of incorporation of ^{3}H-acetate into liver lipids but since large quantities of lipids accumulate in the liver it would appear that in some way the chemical restricts the transport of lipids out of the liver. This storage reached a maximum at about 3 days after dioxin was given and was accompanied by a significant increase in the incorporation of ^{14}C-leucine into liver proteins. Seven days after dioxin was given the incorporation of ^{14}C-leucine into liver proteins varied considerably between experiments being slightly higher than controls in Experiment 5 and slightly lower in Experiment 4. Weight gain per day showed an opposite trend being lower than controls in Experiment 5 and slightly higher in Experiment 4 but none of these differences was statistically significant. It is possible that the increased synthesis of liver proteins at 3-days was the result of an induction of liver enzymes (11) in response to the dioxin and that the time required for it to decline again varied slightly between experiments.

} But Bruckner gave 25 mg/kg per day for 10 days!

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TABLE 5

Effect of Dioxin on Lipid and Protein Synthesis 3 and 7 days after Dosage

	3 days			7 days		
Dioxin oral dosage (µg/kg)	0	10	S.E.	0	10	S.E.
Number of rats	4	4		4	4	
Initial weight, g[1]	63.0	63.0	0.7	64.0	65.0	1.4
Weight gain per day, g	5.2	4.9	0.4	5.1	4.5	0.3
Liver weight, g	3.78	4.80	0.20*	4.03	4.65	0.30
Liver lipids						
mg/g liver	60	132	25	56	92	19
mg/liver	229	640	138	226	428	28**
DPM ^{3}H/mg liver	15.3	12.1	4.5	21.7	17.5	3.2
DPM ^{3}H/mg lipid	258	98	42*	394	190	62*
DPM $^{3}H \times 10^{3}$/liver	58	58	22	86	82	9
Adipose tissue, flank						
DPM ^{3}H/mg lipid	1.61	1.53	1.33	2.30	1.35	0.53
Liver protein						
mg/gm liver	277	241	14	252	252	13
mg/liver	1046	1157	66	1015	1169	83
DPM ^{14}C/mg protein	135	156	6*	144	152	8
DPM $^{14}C \times 10^{3}$/liver	141	178	7*	145	177	11
Skeletal muscle						
DPM ^{14}C/mg protein	42	42	2	40	39	2

[1] rats on 3-day treatment were dosed 4 days after initial weights taken and were then killed on the same day as 7-day rats.

*P < 0.05

**P < 0.01

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Acknowledgments

We are grateful to Mr. R.C. O'Brien who assisted in the chemical analyses.

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Neue Zurcher Zeitung Number 70/Noon/February 12, 1973

Dioxins-Insidious Poisons in Our Environment

Origin, properties and behavior of polychlorinated dibenzo-p-dioxins

By Hans Paul Bosshardt*

In the large scale production of chlorinated phenols as side products, very toxic polychlorinated dibenzo-p-dioxins are formed which are damaging to the fetus. These impurities in their environmental behavior are similar to chlorinated hydrocarbons: therefore one has to count on accumulations within biological material and consequently on irreparable damages. In the U.S.A. millions of chickens fall victim to these toxins.

In order to keep our environment and our food and nutritional sources free of these dangerous toxins, according to a decree from the Research Institute of Wädenswil, the especially dangerous tetrachlorodibenzo-p-dioxin must not be detectable in plant protection agents and weed destroying agents. It is to be hoped that the Confederated Office of Health will establish similar specifications for other polychlorinated phenols and all compounds produced from them which are broadly used outside of agriculture in industry and technology, for example in wood protection or that their further application will be forbidden on the basis of the toxic substances law.

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Reliable analytical proof of polychlorinated dibenzo-p-dioxins is extremely difficult and requires the use of modern devices (gas chromatography coupled with masspectrometry) which cannot yet be introduced into the municipal and Cantonal laboratories probably for financial reasons. In order to protect human and animal life and health, and especially in order to prevent possible birth defects, legal-industrial and technical steps are urgently required.

On a worldwide basis, residues of chlorinated hydrocarbons which are used as insecticides (DDT etc.), fungicides (HCB), lubricants and insulation materials (PCB) or as solvents can be detected in biological material. By way of preventative measures for protecting possible irreparable environmental damages, the use of such substances in many countries, also in Switzerland, has been limited or completely forbidden. In addition to these rather well known impurities, with progressive development in analysis technology additional chemical environmental problem substances are beginning to become detectable. With respect to their amount they are indeed less, ecologically however they can be of at least equal significance. By way of an example of such analytically, very difficultly detectable environmental toxin we will describe here polychlorinated dibenzo-p-dioxins in somewhat greater detail which have already caused serious toxic catastrophes although they are present in only relatively minute amounts.

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Impurities of Chlorinated Phenols

In the technical manufacturing of chlorinated phenols, by way of impurities due to the condensation of two molecules, polychlorinated dibenzo-p-dioxins form. This condensation reaction occurs at temperature over roughly 200°C almost quantitatively and in an explosive manner; a few factory installations have already fallen victim to this. Under 180° in technically flawless installations these side products are formed only to a minute extent. Therefore, it was possible for the research agency of Wadenswil to establish for weed killers produced from trichlorophenol based on 2,4,5-T a degree of purity of less than 0.05 ppm of 2,3,6,7-tetrachlorodibenzo-p-dioxin with respect to 2,4,5-T acid; in Germany a similar specification was established (0.1 ppm).

Toxification Catastrophes in Chicken Farms

Due to the dangerous toxicological properties of tetrachlorodibenzo-p-dioxin, strict purity criteria have to be maintained. The acute oral toxicity (LD_{50}) of 2,3,6,7-tetrachlorodibenzo-p-dioxin is:

	LD_{50} g/kg (1 µg = 0.001 mg)
Rats, male	22
female	45
Guinea pigs	0.6 — [illegible]

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The LD_{50} for the lower and for the higher chlorinated dibenzo-p-dioxins is somewhat more favorable:

2,8-dichlorodibenzo-p-dioxin rats orally	5000 mg/kg
2,4,8-trichlorodibenzo-p-dioxin	5000 mg/kg
Hexachlorodibenzo-p-dioxins	1 mg/kg
Octachlorodibenzo-p-dioxin	1000 mg/kg

The acute toxification phenomena with chlorinated dibenzo-p-dioxins is shown in the picture of chloracne: breaking out of the skin, difficult to heal.

Especially dangerous are the polychlorinated dibenzo-p-dioxins due to their high damaging effect on the embryo (teratogeneity): even 0.125 µg/kg of 2,3,6,7-tetrachlorodibenzo-p-dioxin cause phototoxic damages in rats if they are administered daily from the 6th to the 15th day of pregnancy. Also in mice such treatment with 1 µg/kg leads to cleft pallets. The exact evaluation of these and a whole series of further pertinent investigations is in detail quite difficult, still no doubt exists as concerns the fact that tetrachlorodibenzo-p-dioxin is highly teratogenic. The same is also true for the isomeric hexachlorodioxins which when administered to rats in 10 daily doses of 1 µg/kg caused the number of fetuses to drop by one half. Octachlorodioxin, on the other hand, does not seem to be teratogenic. Data concerning experimental investigations on chronic toxicity of chlorinated dibenzo-p-dioxins is lacking.

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The U.S. Food and Drug Administration has available a copius backlog of experience concerning the effect of these substances on poultry. In the years 1957 to 1969 a disease broke out repeatedly in various States in the U.S.A. on chicken farms and many millions of animals fell victim to it. Exhaustive investigations were able to trace this disease back to the chicken feed whose fatty components were made impure with polychlorinated dibenzo-p-dioxins, namely the isomeric hexachloro-dibenzo-p-dioxins. In addition to liver and kidney damages, the autopsy in the animals concerned showed a hydropericardium, that is to say an accumulation of liquid in the heart cavity. This disease was named "chick-edema-disease". Such toxification catastrophes in chicken farms are a warning to be taken seriously because dioxin contaminations do not necessarily have to remain restricted to animal feeds. Also, in addition to the various possibilities of ingesting the toxin through the mouth (orally), probably many possibilities exist that it can also reach the body through the skin (dermally). As an example of this we should point out the toxification of infants due to pentachlorophenol; pentachlorophenol was used in an American drycleaning establishment for treating diapers and sheets and thus led to the death of two newborn infants.

Large Scale Technical Production

The question as to whence and in what way chlorinated dioxins reached chicken feed has not been decisively cleared up because several possibiliti exist for this. Chlorinated phenols and their derivatives are consumed in countless areas of application; by way of examples we will mention here

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the use of pentachlorophenol in wood protection and of trichlorophenol for producing the weed destroying agent 2,4,5-T or the bactericide hexachlorophene. Although no production figures are known, it is easy to estimate that the world production of chlorinated phenols reaches very large dimensions. The production alone of 2,4-D and 2,4,5-T increased in the United States from roughly 15 million kg in the year 1958 to 40 million kg in the year 1966, and for the destruction of the Vietnamese jungle in the years 1962 to 1970 some 22 million kg of 2,4,5-T were also used in addition to an essentially larger amount of 2,4-D. The annual production of sodium pentachlorophenolate in 1969 alone in Japan amounted to over 16,000 tons. The use of pentachlorophenol as defoliant on American cotton fields was mentioned as a possibility of contamination of chicken feed. The most probable source of contamination is considered to be however the treatment of cowhides with pentachlorophenol. This fat adhering to animal tissues is mixed into chicken feed, proved to be very toxic and showed a high content especially of hexachlorodibenzo-p-dioxins. Since technical pentachlorophenol, in addition to some 8% tetrachlorophenol and 0.1% trichlorophenol, contains larger amounts of polychlorinated dibenzo-p-dioxins, these compounds went directly into the fat on the one hand or were able, on the other hand, to be freshly formed in addition from the chlorinated phenols during the melting of the fat.

In addition to these acute oral or dermal toxications with chlorinated phenols and dioxins, one must count on chronic toxications whose cause can scarcely be measured by doctors and patients.

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Only the damages can be measured!

The chemical and physical properties of chlorinated dioxins make such chronic damages very probable. Polychlorinated dibenzo-p-dioxins are stable and principally fat-soluble compounds. Therefore, in the environment they behave according to the pattern of chlorinated hydrocarbons. Tetrachlorodibenzo-p-dioxin was decomposed using ultra-violet or sunlight, whereas octachlorodioxin is stable under these conditions. Aside from this reaction, until today no degradation mechanism is known which can make these dangerous compounds disappear fast enough. 2,3,6,7-tetrachlorodibenzo-p-dioxin is heat-stable up to 700°C and only decomposes completely at 800°C. If paper which was treated with sodium pentachlorophenolate is burnt, the concentration of octachlorodioxin even increases. Tetrachlorodioxin is extremely persistent in various soils and over the course of 160 days is only unessentially degraded. Due to the low water solubility (0.2 ppb) one cannot anticipate its being washed out into the ground water, however, absorption into plants -- even to a minute degree -- could be detected. The absorption of larger amounts, however, is improbable due to the extremely difficult solubility even in apolar solvents (best solvent: o-dichlorobenzene, 0.14%).

These properties favor a broad distribution in the environment so that dioxin contaminations, in addition to the relatively larger contaminations with respect to amounts with chlorinated hydrocarbons in biological material are very difficult to detect. Since dioxins do not all have the same biological effect, the possibility exists that this unmeasurable contamination ecologically is as significant as the measurable one. In

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this sense dioxin-contamination is a prime example of environmental damage: nothing is measurable and damages can appear however at biological foci. The contamination only becomes measurable when the damages are irreversible.

Preventative Measures

In order to prevent such damages, in the near future the technical production of chlorinated phenols and all subsequent products manufactured from them and formulations must be improved in such a way that no more polychlorinated dibenzo-p-dioxins are formed. Should this not be possible, then the use of these preparations should be drastically limited or completely forbidden. In a new Confederated Toxic Substances Law, which is in force now for 1 year, legal bases are provided which make it possible to rapidly control this problem area. The manufacturers of chlorinated phenols will probably lend a hand in bringing about a hygienically satisfactory control and do everything to make their production units inert within a useful amount of time.

It is understood that such problems cannot be solved in Switzerland alone. International cooperation is indispensable and fortunately has already been extensively realized. It would be quite proper however for our country in such matters to lead the way and work out proposals for possible solutions of problems of prevention and restoration of chemical environmental contaminations.

(A pertinent literature index can be made available upon request.)

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[1973] K-66681-(100) Revised

AN ANALYTICAL METHOD FOR DETECTING DIOXIN*

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an extraordinarily toxic substance that is produced as an unwanted side product in the industrial synthesis of 2,4,5-trichlorophenol, an intermediate in the manufacture of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).[1,2] Because of its chemical stability and its lipophilic nature, the possibility exists that TCDD released into the environment could accumulate in food chains. A direct test of the possibility of biologically significant accumulation in animal tissues requires an analytical method able to detect TCDD at levels well below those known to be toxic. The lowest value known for the lethal dose of TCDD is that observed in the guinea pig, for which the single oral dose LD_{50} is 600 parts per trillion (ppt) body weight.[3] Allowing for sublethal toxic effects and providing for a conservative margin of safety, it seems desirable to have an analytical sensitivity of at least one ppt. For a one gram sample this means the method must have a sensitivity of about 10^{-12} grams or one picogram (pg).

The most common method for analyzing chlorinated organic compounds in tissue samples is gas-liquid chromatography (glc) with an electron capture detector. Its limit of detection for TCDD, 10^{-9}

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gram, is inadequate. This method is also susceptible to interference from other compounds and so is not very specific.

Mass spectrometry offers better possibilities. It is highly sensitive and in the high resolution mode of operation it is highly specific. We have previously described a time averaged mass spectroscopic method with an adequate limit of detection.[4] However, full sensitivity could not be realized in most sample types because of interference from DDE (a major degradation product of DDT) and polychlorinated biphenyls (PCB's). In this paper we describe a cleanup procedure that overcomes this difficulty.

Homogenized samples are saponified in alcoholic potassium hydroxide and extracted with hexane. The extract is shaken with sulfuric acid and chromatographed on alumina. Elution with carbon tetrachloride-hexane removes most of the DDE and PCB's. Chlorinated dioxins are then eluted with dichloromethane-hexane. The TCDD containing fraction is further purified by preparative gas-liquid chromatography and analyzed by mass spectroscopy, using a multi-channel analyser (CAT) to average successive scans.

We also report the levels of TCDD found in a limited number of samples of fish and crustaceans from locations in South Vietnam near areas heavily exposed to 2,4,5-T.

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EXPERIMENTAL SECTION

A. Reagents and Apparatus.

1. Hexane: Pesticide grade (Fisher Scientific).
2. Dichloromethane: Reagent grade (Eastman).
3. Carbon tetrachloride: Reagent grade (Merck).
4. 95-97% Sulfuric acid: Reagent grade (Dupont).
5. Sodium carbonate (powdered): Reagent grade (Mallinckrodt).
6. Ethanol: Pesticide grade (Matheson, Coleman and Bell).
7. Activated alumina: Fisher No. A-540, activated at 130°C for 24 hours.
8. Gas chromatograph: Bendix Model 2200 equipped with thermal conductivity detector.
9. Gas chromatographic column: 5% SE-30 on 60/80 Chromosorb W, 2 m x 2 mm (id) stainless steel.
10. Trap for preparative gas chromatography: 150 mm x 1.5 mm (id) glass tube packed with 30 mm of glass wool.
11. Mass spectrometer: Associated Electrical Industries MS-9 double focusing mass spectrometer.
12. A Varian 1024 time averaging computer (CAT), interfaced with the MS-9 as described earlier.[4]

B. Cleanup Procedure for the Analysis of TCDD in Tissue Samples.

1. Weigh the sample and homogenize it with 1.0-1.2 parts EtOH.

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Homogenizer Ig style?

µg #3 10 x 10 mm Tra 200 µm 30 ml tube

10 g. g.l. 1 quart CTAB 2 ml
2 quart tot + 44 66 ml.
1 quart buffer 66 ml 132 ml.
3 mercaptoethanol 110
214 ml.

#6 ethanol wash = µg H. Leaf Done? each time.
(4 times)
dried to dry?

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2. Transfer the homogenate to a round bottomed (RB) flask equipped with a reflux condenser (Teflon tape should be used on the ground glass joint). Spike the sample with approximately 1000 ppt ^{37}Cl TCDD, add 2 parts 40% aq. KOH, and reflux for 2 hrs.

3. Let the solution partially cool and add 1 part hexane.

4. Transfer the solution to a separatory funnel, separate phases and extract with three more identical portions of hexane. Collect the hexane in the original RB flask.

5. Transfer the hexane to the separatory funnel, rinse the RB flask twice with a few ml of EtOH and then twice with a few ml hexane, refluxing solvent each time, and extract the hexane with 1 part 1.0 N NaOH.

6. Extract the hexane four times (or until acid phase is colorless) with 2 parts 95-97% H_2SO_4. Break up emulsions with a few drops of saturated Na_2CO_3 solution.

7. Extract the hexane with 1 part water and add several grams of Na_2CO_3 to the hexane.

8. Filter the hexane through a column of Na_2CO_3 (100 mm x 10 mm id for 300 ml hexane). Prewash Na_2CO_3 with several ml of hexane.

9. Concentrate the hexane (Snyder column) to 3-4 ml.

10. Chromatograph the hexane residue on a column of activated Al_2O_3 (50 mm in a 5 mm disposable pipette). Do not prewash column. Elute with 12 ml of 20% CCl_4 in hexane, then 1 ml of hexane, and finally 4 ml of 20% CH_2Cl_2 in hexane.

11. Concentrate the 20% CH_2Cl_2 fraction carefully to about 50 μl, add 100-200 μl benzene and concentrate

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again to 20 μl.

12. Add a few μg of m-terphenyl in benzene to the residue and preparatively chromatograph. The retention time of m-terphenyl relative to that of TCDD should be determined beforehand and used to make certain that the TCDD collection is carried out at the right retention time.

13. Elute the glc trap containing TCDD with 60 μl followed by 10 μl of benzene. Measure the total amount of eluant collected and calculate the fraction size in μl for the planned number of fractions (typically ten).

14. Prepare the fractions for TCDD analysis in the sample tubes described previously.[4] Add a known amount of TCDD to three or more fractions for quantitation of any TCDD observed. The amount of TCDD added per fraction for quantitation should be approximately 3-4 times the amount expected to be present.

15. Analyze the fractions with the MS-9/CAT. Typical conditions are: source 220°C, resolution 10,000 (based on a 10% valley between peaks), trap current 1.0 mA (rhenium filament), electron multiplier 700, ionizing voltage 70 eV, time averaging at four scans per second.

16. Measure peak heights at m/e 321.894. Compute the quantity of TCDD (grams x 10^{-12}) present in the fractions to which TCDD has not been added from the ratio of their mean peak heights to the mean peak heights found with added TCDD.

17. Repeat steps 14-16, but add ^{37}Cl TCDD and measure peak heights at m/e 327.885 in order to compute the amount of ^{37}Cl TCDD recovered. Calculate the recovery

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through the complete cleanup procedure based on the amount of ^{37}Cl TCDD added to the sample at the beginning of the cleanup.

18. Correct the quantity of TCDD computed in step 15 by the recovery factor obtained in step 16 to give the final result.

C. Sample Collection.

Freshly caught fish and crustaceans were collected in South Vietnam in August and September 1970 from local fishermen. The samples were homogenized with a meat grinder, placed in acetone-rinsed glass bottles with aluminum foil lined caps, and immediately frozen in dry ice. Later on the same day, samples were placed in a Linde LR-35 liquid nitrogen refrigerator where they remained until analysis. Water blanks were present in the liquid nitrogen refrigerator throughout the storage period and were analyzed with the samples. Fresh Cape Cod butterfish (Poronotus tricanthus, family Stromateidae) were obtained from a local market, homogenized, and kept at -20°C until analysis. Domestic beef livers were obtained and treated similarly.

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RESULTS

A. Methodology.

The mass spectra of natural and ^{37}Cl TCDD are shown in Fig. 1. The most intense signal for natural TCDD occurs at m/e 321.894 (nominal m/e 322), corresponding to the isotopic isomer with one atom of ^{37}Cl and three atoms of ^{35}Cl. The natural abundances of the Cl isotopes are 75.53 and 24.47 percent, respectively. The

observed spectrum for the synthetic ^{37}Cl TCDD corresponds tc an isotopic purity of 95.5 percent ^{37}Cl, the same as the value claimed by the manufacturer of the NaCl used in the synthesis of the labeled TCDD. The synthetic ^{37}Cl TCDD contributes only 0.042 percent as much to the peak at m/e 322 as to its most intense signal at m/e 328. The contribution at m/e 320 is even lower, by a factor of nearly 100. This allows an excess of ^{37}Cl TCDD to be added to each sample before cleanup without interfering with analysis of natural TCDD at m/e 322 and 320. The addition of ^{37}Cl TCDD provides a carrier and makes possible the calculation of absolute recoveries.

An alumina chromatography step has been developed which, when combined with the cleanup steps described previously,[4] makes possible the measurement of picogram quantities of TCDD in samples initially containing more than a million-fold excess of DPE and PCB's. Fig. 2 shows the effectiveness of this procedure.

The calculation of TCDD levels described in steps 14-16 of the experimental section assumes a linear relationship between peak height and amount of TCDD present in any given sample. Fig. 3 demonstrates that the response is indeed linear over the full range of TCDD amounts introduced into the MS-9 in the course of the analyses reported here.

The reproducibility and overall recovery of the complete analytical procedure is illustrated in Table 1. A sample of beef liver was homogenized and divided into three portions each of which was then spiked with 20 ppt TCDD and 1000 ppt ^{37}Cl TCDD. The three samples were independently put through the cleanup procedure up to the glc step. Each sample was then split into three portions before preparative glc and mass spectrometric analysis, giving rise to a total of nine separate values for the recovery of both TCDD and ^{37}Cl TCDD. The average recovery was 34 $\pm$ 7 percent for TCDD and

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27 ± 5 percent for ^{37}Cl TCDD. When the slight background signal at m/e 322 in an unspiked sample of the same liver is taken into account, the calculated recoveries from the spiked samples become even more nearly equal. Experiments performed separately with each individual cleanup step established that the step with lowest recovery is preparative gas-liquid chromatography.

We conclude from these and other controls that the present analytical method provides the sensitivity and reproducibility required for biologically meaningful analyses of animal tissue samples. The method makes possible investigations of such samples at levels approximately ten thousand times lower than those reported heretofore.[5]

B. Observed TCDD Levels.

Signals at m/e 320 and 322 were conspicuously present in each of the fish and crustacean samples from Vietnam. The calculated levels of TCDD, summarized in Table 3, range from 18 ppt to 814 ppt, based on total wet body weight.

No peak was observed at m/e 320 or 322 with Cape Cod butterfish. The background signal corresponded to a level of ≤3 ppt of TCDD. No peaks were observed in water blank samples present in the liquid nitrogen refrigerator throughout the sample collection and storage period.

Confirmation that peaks observed at m/e 320 and 322 are in fact produced by TCDD is routinely provided by the criteria outlined in part A of Table 2. All three of these criteria are met by the mass spectra from each of the Vietnamese samples.

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The additional confirmatory procedures listed in part B of Table 2 were carried out on a sample of Vietnamese fish. This sample, carp from the Dong Nai River, exhibited a mean TCDD level of 540 ppt. The mass spectrum in the region m/e 322 is shown in Fig. 4. The compound observed in this fish behaved identically to TCDD in each of the three additional confirmatory tests. We consider it extraordinarily unlikely that this compound is anything other than a tetrachlorodibenzo-p-dioxin. In contrast to the significant amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin known to have been disseminated as a contaminant of 2,4,5-T,[1] we know of no mechanism by which other isomers of TCDD might have been introduced into the Vietnamese environment.

We have ruled out the possibility that the TCDD was formed during the saponification step of the cleanup procedure. It is known that chlorophenols and chlorophenoxyphenols can form dioxins under alkaline conditions at high temperature.[1] A sample of Dong Nai catfish was worked up omitting the saponification step. The sample was digested in concentrated sulfuric acid and carried through the rest of the usual cleanup. The TCDD level, 304 ppt, was somewhat lower than the 522 ppt observed after the complete cleanup but the difference was not significant.

The locations from which the Vietnamese samples were obtained are designated in Fig. 5. The letters correspond to those in Table 3. Areas heavily treated with 2,4,5-T before its use was ordered discontinued in April 1970 are shown as stipled. The number of samples is not adequate to permit reliable conclusions concerning the differences between various locations and species, although this certainly should be a subject of future studies.

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Considering the limited number of samples we have analyzed and the fact that they were collected two and a half years ago, it does not seem appropriate to attempt any detailed evaluation of the possible toxicological significance of our results. Such discussion is made even more difficult by the complexity and incompleteness of the existing toxicological data. However, in order to provide perspective for such discussion, a tabulation of some of the principal toxicity data on TCDD is presented in Table 4. It may be noted that guinea pigs consuming their weight of food contaminated with TCDD at a level of 600 ppt would have ingested a quantity corresponding to the lethal dose. In contrast, a far greater quantity of TCDD is required to reach the LD_{50} cited for rats. The table shows that teratogenesis in the rat occurs at doses substantially lower than those required to kill.

Feeding studies in monkeys show that dioxin poisoning is cumulative.[6] Various levels of a toxic fat known to contain chlorodioxins were incorporated into the daily diet of Macaca mulatta monkeys. As pointed out by the investigators, the mean survival time depended inversely on the daily dose. A plot of their data (Fig. 6) conforms rather well to the relation (mean survival time) = K/(daily dose) + K', where K and K' are constants corresponding, respectively, to the accumulated lethal dose and to the lag time between the accumulation of this dose and the time of death. No departure from this relation is seen even at the lowest level of toxic fat tested, where the mean survival time was 445 days. The importance of this result is that repeated intake of quantities of TCDD individually equal to only a small percentage of the single oral dose LD_{50} may over time cause serious poisoning. Unfortunately, the LD_{50} for TCDD in these primates cannot be computed since all of the animals died (5/5) even at the lowest dose level and the concentration of TCDD in the toxic fat employed has not been definitely established.

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In South Vietnam itself we have little information regarding the possible occurrence of toxic effects of TCDD in humans nor has it been established whether or not measurable levels of TCDD have accumulated in exposed populations. Certainly, it should be pointed out that while we were in South Vietnam in 1970, the medical member of our group, Dr. John Constable, Professor of Surgery at Harvard Medical School, did not encounter evidence of any severe and widespread unusual illness in visiting Can Gio and several other villages or in discussions with officials of the South Vietnamese Ministry of Health. However, it was felt that certain indications in birth statistics ought to be investigated further for possible connections with herbicide exposure.[11] It is of obvious interest to survey appropriately chosen populations in South Vietnam more closely, especially if TCDD residues should be found in human tissue samples.

Finally, turning from questions of environmental toxicology to the biological mechanisms of action, we note that TCDD seems to be particularly toxic to proliferating tissues, as suggested by its effects on spermatogenesis and hematopoiesis and its apparent toxicity to the intestinal epithelium[6] and the thymus.[12] These indications are consistent with the effects of a mitotic poison, such as TCDD is known to be in the African blood lilly[10] and possibly in Drosophila melanogaster.[13] We are led by these observations to speculate that TCDD may be able catalytically to disrupt microtubules, the subcellular elements of which spindle fibers are constructed and which are ubiquitous in their structural roles in cell extension and cell movement.

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SUMMARY

1. A procedure has been developed for the reliable detection of TCDD in animal tissues down to levels approaching one ppt. It makes use of chemical cleanup, preparative gas-liquid chromatography and analysis by time averaged high resolution mass spectroscopy.

2. A limited number of fish and crustacean samples was collected in South Vietnam in 1970 near areas heavily exposed to the herbicide 2,4,5-T. TCDD was detected in these samples at levels ranging from 18 to 814 ppt. TCDD was not detected in a sample of Cape Cod butterfish used as a control.

3. These results suggest that TCDD may have accumulated to biologically significant levels in food chains in some areas of South Vietnam exposed to herbicide spraying.

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FIGURE LEGENDS

Figure 1. Mass spectra of TCDD (A.) and $_{37}^{37}Cl$ labelled TCDD (B.). The isotopic purity of the ^{37}Cl is 95.5%. The asterisk denotes an impurity. The multiplicity of lines associated with each major molecular species results from the presence of various isotopes of Cl and C.

Figure 2. Mass spectra showing reduction of DDE and PCB levels in fish residue by means of alumina chromatography. Following the sulfuric acid cleanup step, the residue in hexane is added to a column of activated alumina. The trace in part A of the figure is from the material eluted by 20% CH_2Cl_2 in hexane after the column was first eluted with 20% CCl_4 in hexane. The trace in part B was obtained from a similar 20% CH_2Cl_2 in hexane elution after the column was first eluted with 1% CH_2Cl_2 in hexane. Elution with 1% CH_2Cl_2 in hexane was reported to be effective in reducing the amount of PCB residues (Porter and Burke, 1971). Elution with 20% CCl_4 is clearly even more effective and was routinely used in obtaining the results reported here.

Figure 3. Linearity of response for TCDD in the presence of beef liver residue. The TCDD values are the amounts introduced into individual runs on the MS-9.

Figure 4. TCDD signals observed in fish samples. A. Vietnamese carp plus 60 pg TCDD. B. Vietnamese carp. C. Cape Cod butterfish. The wet weight of fish represented in run A and run B is 0.18 g. In C it is 0.16 g.

Figure 5. Map showing sampling sites in relation to rivers and principal sprayed areas. Sites A and B are located on the Dong Nai River, site C is on the Sai Gon River, and site D is on the coast at Can Gio. Sprayed areas are depicted only within the region bounded by the dashed lines (____).

Figure 6. Mean survival time of monkeys fed toxic fat (Allen and Carstens, 1967) plotted against the reciprocal of the percent of toxic fat present in the diet.

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e address presented at the 25th annual meeting of the Southern Weed Society at Dallas, Texas, Jan. 18, 1972)

Teratological potential of 2, 4, 5-T

By James G. Wilson, Ph.D.
Children's Hospital Research Foundation and
Departments of Pediatrics and Anatomy
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The herbicide 2,4,5-T has received more notoriety and probably has been the cause of more public concern than any other pesticide except the insecticide DDT. Since early in the Vietnamese war, 2,4,5-T, together with several other herbicides, was extensively used as a defoliant to reduce the likelihood of ambush in areas where dense undergrowth in the forests and swamplands of Vietnam favored guerilla warfare. During June and July of 1969, South Vietnamese newspapers reported that there had been an increase in human birth defects in some parts of the country. The suggestion was made that this increase was somehow related to the use by the United States Armed Forces of defoliant chemicals.

These reports of possible teratogenic effects in Vietnam might have gone unnoticed had they not been followed in October, 1969, by the announcement of Dr. Lee A. DuBridge, then the President's Science Advisor, that use of 2,4,5-T in the United States was soon to be restricted by certain government agencies. The announcement was a consequence of the release a few days earlier of the results of a study of a number of pesticides and industrial chemicals by Bionetics Laboratories which indicated that mice treated during early pregnancy with large doses of 2,4,5-T gave birth to malformed offspring.

Dr. DuBridge's announcement, together with the Vietnam reports that human birth defects had possibly resulted from exposure of pregnant women to this herbicide, had widespread repercussions. During the following months a veritable epidemic of high level discussions occurred in the form of: government-sponsored panels of experts; commissions set up by scientific societies; conferences of scientists from universities, chemical industries and the government; and even a subcommittee of the U.S. Senate. These sundry groups discussed the matter at length but were unable to resolve the main issue of whether 2,4,5-T, as currently produced and used, represented a real risk for human pregnancy.

The question remained unanswered in January, 1971, at which time it was inherited by the newly formed Environmental Protection Agency (EPA). During the previous year government restrictions had been placed on the use of 2,4,5-T, both for military purposes in Vietnam and for several types of use in the United States that, it was thought, might result in exposure of pregnant women. Manufacturers of 2,4,5-T as well as agricultural interests that used it were entitled under law to ask for scientific review of and/or public hearings on the bases for the restrictive actions. Two manufacturers, The Dow Chemical Company and Hercules Incorporated, had already requested a scientific review, several months before EPA officially came into being.

One of EPA's earliest functions was to activate in January, 1971, a Scientific Advisory Committee which was charged with responsibility to review all available evi-

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dence on the teratogenic potential (capacity to cause birth defects) of 2,4,5-T and to recommend any courses of action that would seem to be indicated by the scientific data. A nine-man committee was selected from a list of scientists submitted by the National Academy of Science, and regarded by the Academy as having appropriate qualifications.

The Committee was allowed only four months in which to collect and evaluate all information that had any scientific credence and to prepare its report. This was a formidable job in view of the thousands of pages of research data, expert opinion, and survey results that had accumulated, particularly during the previous two years. Nevertheless, in May, 1971, a 75-page report* was submitted to William D. Ruckelshaus, who had, a few months earlier, been appointed Administrator of the EPA. The report represented a concensus of agreement reached by all of the nine-man Committee except for one member. The dissenter was Dr. Theodore Sterling, the only member not a qualified bio-medical scientist, who asked to append a lengthy list of objections and exceptions to the main report.

Almost immediately the report was leaked to two scientific news magazines *(Nature* and *Science)* in spite of the fact that the regulations under which the Committee operated specifically prohibited any release until definitive action had been taken by the EPA. Highly critical magazine articles appeared during the summer based almost exclusively on the objections and exceptions of the one dissenting member. No more than passing reference was made to the conclusions and recommendations of the eight toxicologists, teratologists, botanists and biochemists who had concurred in the main report.

By all appearances the person or persons behind these distorted magazine accounts hoped to destroy the credibility of the Report and arouse prejudice in the minds of scientists and the public before the EPA had an opportunity to examine it in any objective way, much less take carefully considered action. The only action EPA has taken to date, has been a news release by Mr. Ruckelshaus on August 9, 1971, stating, "A public hearing will be held in the fall to obtain additional facts on 2,4,5-T while the cancellation order continues on the use of the herbicide on food crops grown for human consumption..." Ruckelshaus was further quoted as saying that "while the Advisory Committee Report significantly adds to an understanding of 2,4,5-T, questions remain as to the balance between benefits and risks from use of the herbicide." The news release noted that the Committee was not charged with assessing benefit-risk factors and that a public hearing would be desirable to resolve this issue. No public hearing has yet been scheduled.

If it was the intent of the persons who leaked the report to discredit it before its legitimate release, this objective was only partially achieved. In the first place the magazine reports were so flagrantly biased that even readers unfamiliar with the subject could hardly miss the selectivity used in describing the contents of the report. The careful evaluation of data and justification of conclusions presented by the Advisory Committee were neither quoted nor discussed. On the other hand, most of the objections and criticisms of the one dissenting committee member were reported in as favorable a light as the writer could manage, despite the fact that these objections were based on little more than personal opinions and subjective reactions. One of several unfortunate consequences may be that *the reliability of scientific journalism* will remain in doubt in the minds of many readers. Although letters were written to the magazine principally involved, objecting to the overt bias and unfairness of the articles on the 2,4,5-T Advisory Committee Report, the only letter yet to be published was that signed by the entire membership of the Council of the Society of Toxicology. The protest of a panel so highly qualified to pass judgment could hardly be ignored, nevertheless, the letter of the Council was delayed several months before it was printed. *(Science,* Nov. 5, 1971).

But what about the main issue—is 2,4,5-T really teratogenic? The answer is *yes*, but so are hundreds of other commonly used drugs, plant products and environmental chemicals (Table 1). These compounds were given to pregnant laboratory animals in doses usually greatly in excess of that equivalent to the human therapeutic dose, in the case of drugs, or of the likely exposure level, in the case of environmental chemicals. This list of chemicals now known to be teratogenic in rats, mice or rabbits is so

*Copies of the Report of the 2,4,5-T Advisory Committee are available by writing:
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Washington, D.C. 20460

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Table 1—Types of Drugs and Other Chemicals That Have Been Shown To Be Teratogenic* in One or More Species of Laboratory Mammal

Salicylates **(e.g., aspirin, oil of wintergreen)**

Certain Alkaloids **(e.g., caffeine, nicotine, colchicine)**

Tranquilizers **(e.g., meprobamate, chlorpromazine, reserpine)**

Antihistamines **(e.g., buclizine, meclizine, cyclizine)**

Antibiotics **(e.g., chloramphenacol, streptonigrin, penicillin)**

Hypoglycemics **(e.g., carbutamide, tolbutamide)**

Corticoids **(e.g., triamcinolone, cortisone)**

Alkylating agents **(e.g., busulfan, chlorambucil, cyclophosphamide, TEM)**

Antimalarials **(e.g., chloroquine, quinacrine, pyrimethamine)**

Anesthetics **(e.g., halothane, urethan, nitrous oxide, pentobarbital)**

Antimetabolites **(e.g., folic acid, purine and pyrimidine analogues)**

Solvents **(e.g., benzene, dimethylsulfoxide, propylene glycol)**

Pesticides **(e.g., 2,4,5-T, carbaryl, captan, folpet)**

Industrial effluents **(e.g., some compounds of Hg, Pb, As, Li, Cd)**

Miscellaneous **(e.g., trypan blue, Tryparanol, Diamox, etc.)**

*Teratogenic effects were usually seen only at doses well above therapeutic levels for the drugs, or above likely exposure levels for the environmental chemicals.

Table 2—Pesticides Claimed To Be Teratogenic in Laboratory Mammals

Captan and Folpet	**rabbits and mice**
Carbaryl (Sevin)	**mice and dogs**
Dieldrin, Chlordane and Keptone	**mice**
Diquat and Paraquat	**rats**
Various organomercury compounds	**mice**
2,4,5-T* and 2,4-D	**rats and mice**

*Teratogenic effects may have been caused by an impurity.

extensive that it is quite natural to ask if not all chemicals might be damaging to embryonic animals under the right conditions. As a matter of fact, investigators in the field of teratology tend to assume that this is indeed the case. One of the widely accepted principles in the field is called Karnofski's Law, which states, "that any drug administered at the proper dosage, at the proper stage of development, to embryos of the proper species, will be effective in causing disturbances in embryonic development."

Although Karnofski's Law specifies only drugs, it undoubtedly holds true for many other, if not all, types of chemicals. All pesticides would certainly be suspect because they, like antibiotics, sulfonamides, antitumor compounds and immuno-suppressive drugs, were designed and developed either to kill living organisms or to suppress their metabolism and growth. Three years ago a survey of the literature (Table 2) revealed that several pesticides had at that time already been reported to be teratogenic in mammals. Today the list would be at least twice as long. Thus it is probably safe to assume that most pesticides given in high dosage to pregnant mammals would, if they did not kill the mother first, interfere with the growth and/or metabolism of the embryos. No biological system is known to grow faster than the mammalian embryo during its early differentiation and organ formation.

Why then, if most pesticides are likely to be teratogenic at high doses, did so much excitement follow the report in 1969 that 2,4,5-T at high doses caused malformations in mice? The answer is not entirely clear, but it probably relates to all of the following conditions: 1) the high level of emotion that surrounded all aspects of the Vietnam War, 2) the unsubstantiated report that human birth defects had resulted from defoliation in Vietnam, followed within a few weeks by the report of the teratogenic effects of 2,4,5-T in mice, 3) the widespread public concern about many aspects of environmental change and their effects on "ecology", 4) over-zealous and sometimes irresponsible efforts by some agencies and individuals to influence public opinion for personal gain, or for sincere but ill-founded reasons.

Now I would like to speak directly to the subject of the teratogenic potential of 2,4,5-T. The Advisory Committee agreed in a ratio of 8:1 that acceptable data on the embryotoxicity of 2,4,5-T were available for 6 mammalian species; namely, mouse, rat,

5843

hamster, rabbit, sheep, and rhesus monkey. None of these showed adverse effects at a dosage of 40 mg/kg/day during the period of organogenesis. The mouse was the most sensitive of the species studied, showing a low level of teratogenicity, mainly cleft palate, at 100 mg/kg/day given throughout organogenesis. Hamster and rat required higher dosage to obtain comparable effects. It was thought likely that all species could have been caused to show embryotoxicity if 2,4,5-T dosage had been raised high enough. For example, pregnant rats given a single dose of 200 mg/kg on day 9, the most susceptible time for teratogenesis in this species, caused modest increases in the percentage of malformed suriviors as well as in percentage of intrauterine death.

The dioxin contaminant 2,3,7,8-tetrachlorodibenzo-paradioxin (TCDD) has also been shown to have a low teratogenic potential at doses in excess of 0.001 mg/kg. This dosage level is virtually impossible to achieve with currently produced 2,4,5-T, which rarely exceeds 0.5 ppm and usually contains much less.

Careful scrutiny of the reports of adverse effects on human reproduction after use of 2,4,5-T as a defoliant in three separate locations, namely, Vietnam; Globe, Arizona; and Swedish Lapland, yielded no acceptable evidence that the alleged effects were related to exposure to 2,4,5-T. It was concluded that, as currently produced and as applied according to the regulations in force prior to April, 1970, 2,4,5-T represented no hazard to human reproduction.

This was the situation as of May 7, 1971, when the Advisory Committee submitted its report. Has anything happened in the meantime that would make it necessary or desirable to change this conclusion?

New data from Germany (Neubert and Dillman) reported that one strain of mice showed significant increase in cleft palate incidence at a dose of 45 mg/kg of pure 2,4,5-T on days 6 through 15 of gestation. An increase in cleft palate was also noted after treating such mice with doses of TCDD exceeding 1 μg/kg. More significantly, potentiation of the teratogenic effects of 2,4,5-T by TCDD were obtained, *but only when threshold or higher doses of both substances were used.* This demonstrated that a TCDD content of <1 ppm was unlikely to contribute to the embryotoxic effect of 2,4,5-T, even in this highly sensitive strain of mouse.

Canadian workers (Khera and Ruddick) have shown that Wistar rats given 0.5 μg/kg of TCDD on days 6-15 produced young with some embryotoxic signs. *No evidence of dominant lethal mutations* was seen in surviving males after any dose.

Kearney and associates at the U.S. Department of Agriculture have reported that TCDD is immobile in soils, not readily taken up by plants, subject to photodecomposition, persistent in soils and slowly degraded in soils to polar metabolites. "Subsequent studies revealed that environmental contamination by TCDD is extremely small and not detectable in biological samples."

Woolson and associates from several governmental agencies found no dioxin residues in eagle samples from various geographical regions of the U.S. or in soil samples after application of 2,4,5-T in total amounts of 912 lb/acre over the span of 7 years.

These and other data have become available since the Advisory Committee submitted its report. The new information in large measure represents refinements and moderate extensions of information that was already available, or could reasonably be deduced from data examined by the Committee. In any event, the new data do not indicate that a single one of the recommendations made in the original would need to be changed if the report were re-written today.

This is probably reflected in a news item in the *Ottawa Journal* of Dec. 6, 1971, to the effect that the Canadian Government has begun to relax the restrictions placed on the use of 2,4,5-T. It was stated that "Starting next year, manufacturers who produce 2,4,5-T with a dioxin content of less than 0.5 ppm will be able to sell their products without the warnings—now required on all 2,4,5-T labels." An interesting commentary on the situation in the United States is contained in the same article. "The controversy about the applicability of animal tests to humans still rages, particularly in the U.S. where the herbicide's use has political overtones." In fact, the article makes clear that the Canadian Government is ready to adopt essentially all of the recommendations set forth in the Report of the 2,4,5-T Advisory Committee. Unofficial reports indicate that restrictions on the use of 2,4,5-T may also be relaxed in 1972 in Sweden and Finland.

Thus the old adage about "a prophet without honor in his own country-" seems to have been once more exemplified in the Report of the 2,4,5-T Advisory Committee.

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TCDD residue disappears

A toxic contaminant that once appeared in the pesticide, 2,4,5-T, was not detected in recent studies of soil samples and bald eagle tissue and so does not appear to be a residual threat to wildlife.

The contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), can be formed during manufacture of some chlorinated phenols if temperatures above safe limits are used (AGR. RES., October 1971, p. 8). Prior to 1969, traces of TCDD were found in some samples of the herbicide 2,4,5-T.

While regulations and industry quality control have been established to eliminate the threat of TCDD contamination in pesticides, chemist Edwin A. Woolson and technician Peter D. J. Ensor of ARS investigated the possibility that TCDD residues from old, extremely heavy 2,4,5-T applications might still pose a threat to wildlife.

The research was done in cooperation with William L. Reichel, chemist with the U.S. Department of Interior, Patuxent Wildlife Research Center, Laurel, Md., and Alvin L. Young, formerly a project scientist with the U.S. Air Force, Eglin Air Force Base, Fla.

They analyzed soil samples from experimental plots of Lakeland sand in Florida that had received massive doses of 2,4,5-T by aerial application during an 8-year period from 1962 to 1970, a period during which TCDD traces of up to 40 parts per million (ppm) had been detected in commercial 2,4,5-T. Analysis for TCDD residues bega in 1970.

A total of 947 pounds of active 2,4,5-T pe acre was applied over a 3-year period to th Lakeland sand. This is a massive dose whe compared to normal application rates fc brush control on grazing land of 2 pounc per acre, or 6 pounds in a 3-year spar Using analysis techniques capable of de tecting less than 1 ppm of TCDD, the sc entists found no residues in 3-foot co samples.

No TCDD residues were found at the low limit of detection—0.05 ppm in eagle tissu Bald eagle tissue extracts were obtaine from 19 carcasses collected in 15 states widely separated as Alaska, Maine, Florid and Missouri. Eagles were used as repr sentatives of the top of a food chain.

The scientists suggest several reaso why no TCDD was detected in either t soil or eagle tissue samples. For exampl dissipation from the soil may have result from microbial degradation, photodecor position, volatility, and/or wind erosion. the case of the eagle tissue samples, t results suggest that TCDD residues fro past pesticide applications were not ava able to enter the food chain.

These results, combined with the toleran limit of 0.1 ppm TCDD assures freedc from health hazards due to TCDD contar nation.

* From October, 1972 issue of *Agricultural Research.*

5845

C.7.0 Summary of Toxicology Studies with 2,4-D in Livestock, June 1973.

As discussed in Section C of the petition submitted by the Industry Task Force on Phenoxy Herbicide Tolerances in December 1967, no effect was produced by repeated oral doses of 2,4-D salt and ester formulations at 50 mg/kg in cattle and 100 mg/kg in sheep, even when administered five days per week for as long as two years. Levels of 250, 500 and 1000 mg/kg caused anorexia and resulted in death after several repeated doses. The following tables summarize studies by USDA with various phenoxy derivatives in livestock. (Complete reports of the USDA studies can be found in Sections C.1, C.2 and C.3 of the December 1970 amendment and in Section C.7.1 and C.7.2 of this June 1973 amendment.) These and other studies with 2,4-D in livestock are also summarized in Section C.0.2 of this June 1973 amendment.

The effects of exaggerated rates of intake were studied by USDA and by Erne in Sweden (C.7.2 and C.7.3). In the USDA study, administration of the 2-ethylhexyl ester of 2,4-D as an emulsifiable concentrate formulation or as technical material at 250 mg/kg/day for 9 to 56 days caused ill effects in all seven animals after 3 to 14 doses and death in one calf after 14 doses and in two sheep biopsied after 17 and 29 doses. A significant increase in plasma magnesium:calcium ratio was noted in the animals that died. These animals also had increased blood urea nitrogen (BUN) levels, and pathological examination at necropsy showed kidney damage and swollen, blood-engorged thyroids from this high dose rate.

In the Swedish study, various effects were observed in pigs given 2,4-D triethanolamine salt or 2,4-D butyl ester at doses of 50 to 300 mg/kg/day for 5 to 103 days (C.7.3). Lesions

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were observed mainly in the digestive tract and the respiratory and excretory organs. Persistently high 2,4-D plasma levels (200 - 400 µg/ml) were found in animals which developed definite signs of poisoning indicating that their threshold level had been exceeded. On the other hand, 2,4-D plasma levels declined to about 10 µg/ml within 24 hours in animals tolerating repeated administration of 50 mg/kg/day.

The 2,4-D amine was also administered at 500 ppm in the diet of pigs (equivalent to 20 mg/kg/day) for up to 12 months. Growth rate was irregular and depressed and locomotory disturbances developed. This exaggerated dietary level also caused difficulties in pregnancy and parturition in the one sow studied, and the piglets were underdeveloped. Surviving piglets fed 500 ppm 2,4-D in the total diet for 35 weeks grew more slowly than controls and also exhibited locomotory disturbances. The 2,4-D plasma half life was highly variable in the pigs in these studies compared to the normal value of 12 ± 2 hours obtained after single oral doses of 50 or 100 mg/kg (C.6.1).

In the USDA studies, 2,4-D salt and ester formulations were administered by drench or in gelatin capsules five days per week for prolonged periods. Although not representative of ingestion of declining residues of 2,4-D in treated forage, they show that spraying pasture and rangeland with 2,4-D for control of weeds and brush presents no hazard to livestock. As discussed in Section D.5.0 of this June 1973 amendment, residues of 2,4-D are not expected to exceed 300 ppm in grass immediately after spraying at 2 lb/A. (Brushy areas requiring higher rates of application generally contain little or no grass and afford poor grazing for livestock.) The residues in grass decrease rapidly to less than 10 ppm within a few weeks after treatment, depending chiefly on

geographic location. This residue amounts to a level of about 1 mg/kg/day in livestock ingesting 3% of their body weight daily on a dry weight basis.

The lack of hazard to livestock from residues in grass was also demonstrated in feeding studies conducted by USDA and The Dow Chemical Company. Phenoxy herbicides were fed at levels up to 2000 ppm in the total diet of cattle and sheep for four weeks prior to slaughter and sampling of tissues for residue analyses. Based on measured feed intake, individual animals ingested 2,4-D at 55 to 62 mg/kg/day continuously for four weeks without apparent ill effect. Similar levels of 2,4,5-T and silvex in the diet caused anorexia in some animals. (Section D.6.4 of this June 1973 amendment is a complete report of this study in cattle and sheep and of a similar study at up to 1000 ppm in dairy cows. It also shows the residues found in milk, cream, muscle, fat, liver and kidney of animals during ingestion and following withdrawal from the herbicides.)

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C.6.0 Summary of Metabolic Studies with 2,4-D in Animals, June 1973.

A review of available information on metabolism of 2,4-D and other phenoxy herbicides in animals was submitted as part of a supplement to PP 8F0670 in September 1968. Work at Cornell indicated that phenoxy herbicides were rapidly excreted unchanged in the urine of ruminants (Ref. 42, 43, 48, 49, 51, 52). Work at Oregon State University in rats showed that maximum residues occurred in tissues 6 to 8 hours after a 1 mg dose and 8 to 17 hours after an 80 mg dose with highest levels in kidney (Ref. 50). Traces of a metabolite were detected in liver. Total residues decreased rapidly to less than 0.02 ppm in all tissues except the stomach within 16 hours after a 1 mg dose. Elimination was also rapid following subcutaneous injection of 2,4-D, its butyl and isooctyl esters at 100 mg/kg in mice by Bionetics Laboratories (Ref. 53). The butyl ester was hydrolyzed more rapidly than the isooctyl ester. Only 5 to 10% of the 2,4-D remained in the carcass after 1 day and no 2,4-dichlorophenol was detected in extracts of whole mice.

Erne in Sweden has studied the distribution and elimination of 2,4-D in rats, calves, pigs and chickens (C.6.1). Single oral doses of 50 to 200 mg/kg were readily absorbed and peak plasma concentrations were attained within 2 hours after dosing in chickens and within 4 to 7 hours in mammalian species. A dose of 100 mg/kg gave peak plasma levels of about 100 to 200 µg/ml in the species studied. The peak was attained more slowly after administration of the butyl ester in calves and was much lower after administration of the ester to rats. Plasma levels declined and urinary levels increased when rats

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and pigs were given repeated doses of 50 mg/kg/day, except in two pigs which exhibited signs of poisoning at this dose level. A similar decline in peak plasma level was noted in chickens given 2,4-D amine at 300 mg/kg/day.

Erne also studied tissue levels of 2,4-D in pigs maintained on a diet containing 500 ppm of the herbicide and in rats, chicks and chickens given 1000 ppm in their drinking water for 2 months to 2 years. However, the levels found are not representative of normal tissue distribution because the threshold dose was exceeded as evidenced by anorexia and reduced weight gain. Only low levels of 2,4-D and no intact ester were found in tissues of pigs given the butyl ester at 50 mg/kg/day for 1 month. In all cases, the lowest levels were found in fat and muscle with higher levels in liver and kidney. Similar results were found in feeding studies conducted by USDA in calves and sheep (Section D.8.0, June 1973).

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Erne also demonstrated that 2,4-D is bound to plasma protein and that a portion is excreted in the urine in conjugated form (C.6.2). In his method of analysis for 2,4-D in biologic material, the samples were extracted with an organic solvent under acid conditions, followed by solvent partition, thin layer chromatography, or photometric estimation of the color produced with chromotropic acid (C.6.3). However, some conjugates may require alkaline extraction and hydrolysis (D.8.11)

Recent work at Dow in pregnant rats has demonstrated that 2,4-D and 2,4,5-T undergo placental transfer both into and out of the fetuses (C.6.4). Placental and fetal levels were proportional to maternal serum levels in this study and in another with labeled 2,4-D in mice (C.6.5). The radioactivity showed a slight tendency for accumulation in the visceral yolk sac, passed to the fetus and was rapidly eliminated (within 24 hours) from all tissues.

In related work with 2,4,5-T, it was shown that uptake, distribution and elimination depend on the species and on the dose level(see PP 1F1102, Section C.8.0). A single oral dose of 5 mg/kg was excreted rapidly unchanged in the urine of rats and man, but was retained longer and was partially metabolized in dogs. The distribution, metabolism and excretion of 2,4,5-T in rats was much the same following doses of 5 or 50 mg/kg but was markedly different following doses of 100 or 200 mg/kg. Thus detoxification processes may be quite different at high doses such as those which caused effects in teratology studies with 2,4-D and 2,4,5-T (Section C.5.0 of this June 1973 amendment).

A gas chromatographic method has been reported recently for the determination of trace quantities of 2,4-D and 2,4,5-T and their respective phenolic moieties in urine (C.6.6). In rats given 2,4-D at 10^{-5} to 10^{-1} of the LD_{50} (375 mg/kg), the major portion of the administered herbicide was excreted as the unchanged compound and no metabolized residues were detected. Detectable residues of 2,4-D were present in urine within 24 hours after administration of 3.75 µg/kg. Urine samples from occupationally exposed people were found to contain 0.2 to 1.0 ppm 2,4-D by this method.

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Amendment to PP 8F0670 - 2,4-D - June 1973

SECTION C. FULL REPORTS OF INVESTIGATIONS MADE WITH RESPECT TO THE SAFETY OF THE PESTICIDE CHEMICAL

C.0.2 Summary of Toxicology Studies with 2,4-D, June 1973

The following tables and abstract summarize the data available on acute, subacute and chronic toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D) in a variety of species. A more descriptive summary was presented in Section C of PP 8F0670 submitted by the Industry Task Force on Phenoxy Herbicide Tolerances in December 1967. It was repeated in an amendment submitted in December 1970, along with several reports on toxicity studies conducted by USDA with 2,4-D in cattle, sheep and chickens (Sections C.0, C.1, C.2, and C.3). An amendment submitted in September 1971 included a summary of two-year feeding studies in rats and dogs and of a reproduction study in rats conducted by FDA (Section C.4). A published report of the long term studies is included in this June 1973 amendment (Section C.4.1).

Teratology studies were initiated in 1970 as requested in PR Notice 70-8 and a report of a Dow study with several 2,4-D esters was included in the September 1971 amendment (Section C.5.2). Section C.5.0 of this June 1973 amendment summarizes four teratology studies conducted with 2,4-D in rats, hamsters and sheep, followed by published reports of the studies by Dow (C.5.3), Canada Food and Drug Directorate (C.5.4), the Food and Drug Administration (C.5.5) and the U. S. Department of Agriculture (C.5.6). In each case, no effect was produced by administration of 25 to 50 mg/kg/day during organogenesis.

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Amendment to PP 8F0670 - 2,4-D - June 1973

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A review of information on metabolism of 2,4-D and other phenoxy herbicides was submitted in September 1968 and the portion on animals was repeated in Section D.6.1 in December 1970. Section C.6.0 of this June 1973 amendment summarizes a number of additional studies with 2,4-D in animals, including rats, pigs, calves and chickens (Section C.6.1 and C.6.2), pregnant rats (C.6.4), and pregnant mice (C.6.5). These show that 2,4-T is readily absorbed into the bloodstream and is excreted rapidly in the urine relatively quantitatively as unchanged 2,4-D. Placental transfer of 2,4-D occurs both into and out of fetuses.

Residue studies have been conducted in dairy cows, beef calves and sheep which showed that levels of at least 1000 ppm 2,4-D in the total diet were required to cause detectable residues (0.05 ppm) in milk, muscle or fat, while low levels were found in liver and kidney at 300 ppm in the diet (Section D.6.4 of this June 1973 amendment). Low levels of 2,4-dichlorophenol were also found in liver and kidney of ruminants fed 2,4-D, partly in conjugated or "bound" form which require alkaline hydrolysis prior to quantitation (Section D.8.11).

Several methods of analysis have been used recently for 2,4-D and dichlorophenol in biological materials. These include spectrophotometric measurement of the color produced with chromotropic acid (Section C.6.3), cleavage of the ether link with pyridine hydrochloride and gas chromatographic determination of the liberated 2,4-dichlorophenol (Ref. 45, September 1968), and gas chromatography as methyl esters (Sections D.7.3, December 1970; D.8.9 and D.8.10, September 1971; D.8.12, June 1973) or ethyl esters (Section C.6.6, June 1973).

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This amendment to PP 8F0670 also contains a number of additional reports on toxicology studies with 2,4-D salts

and esters in livestock. Section C.7.0 includes summary tables for studies conducted by USDA (Sections C.2 and C.3, December 1970; C.7.1 and C.7.2, June 1973) and a discussion of an extensive study in Sweden (C.7.3). Some animals tolerated repeated daily doses of 50 or 100 mg/kg by capsule or drench without ill effect while others showed symptoms of anorexia resulting in reduced weight gain. Similarly no effect was noted in calves and sheep maintained for 4 weeks on a diet containing 2000 ppm 2,4-D, equivalent to ingestion of 55 to 62 mg/kg/day (Section D.6.4). However, pigs given 500 ppm 2,4-D amine in the diet for 12 months did not gain as well as controls and developed locomotory disturbances (C.7.3).

Toxicity studies with 2,4-D in birds are summarized in Section C.8.0. Repeated oral doses of 2,4-D amine or ester by pipette caused reduced weight gain at levels of 100 or 250 mg/kg/day (C.7.0). No effect was noted at dietary levels of 500 and 2000 ppm for 7 days in cockerels (C.8.2), of up to 1000 ppm for 3 weeks in chicks (C.8.3), and of 50 or 150 ppm for 28 to 48 weeks in laying hens (C.8.4). Continuous ingestion of 500 ppm in feed or 1000 ppm in drinking water for up to 5 months caused renal hypertrophy in chicks and decreased egg production in laying hens (C.7.3 and C.8.5). Spraying of chicken and pheasant eggs with 2,4-D at normal and exaggerated rates had no effect on hatchability in two studies in the U. S. and Canada, (C.8.6 and C.8.7), but was reported to be very harmful in a French study (C.8.8). However, no data were provided on hatchability of untreated chicken and pheasant eggs under the inadequately described conditions used in the latter study.

Formulation	Organism	Dose	Effect	Reference
Triethanolamine	Swine	50 mg/kg	No effect	(a)
Triethanolamine	Swine	500 mg/kg	Lethal	(a)
Butyl ester	Swine	100 mg/kg	No effect	(a)
Triethanolamine	Chicken	300 mg/kg	No effect	(a)
Butoxyethanol ester	Phytoplankton	1 ppm	16% decrease in CO_2 fixation	(b)
Dimethylamine	Phytoplankton	1 ppm (4 hrs)	No effect on CO_2 fixation	(b)
Ethylhexyl ester	Phytoplankton	1 ppm (4 hrs)	49% decrease in CO_2 fixation	(b)
Propylene glycol butyl ether ester	Phytoplankton	1 ppm (4 hrs)	44% decrease in CO_2 fixation	(b)
Butyl ester	Rats	620 mg/kg	LD_{50}	(c)
Unspecified	Trout	3 mg/l*	Lethal 24 hrs	(d)
Unspecified	Trout	2.2 mg/l*	Lethal 48 hrs	(d)
Unspecified	Man	689 mg/kg	Lethal	(d)
Isopropyl ester	Rats	700 mg/kg	LD_{50}	(e)
Propylene glycol butyl ether ester	Bluegill	3 ppm	48 hr TLm	(f)

* mg/l is approximately the same as ppm.

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ACUTE TOXICITY OF 2,4-D (Cont.)

Formulation	Organism	Dose	Effect	Reference
Alkanolamine	Bluegill	435 ppm	48 hr TLm	(g)
Isooctyl ester	Bluegill	9 ppm	48 hr TLm	(g)
Butyl ester	Bluegill	1 ppm	48 hr TLm	(g)
Isopropyl ester	Bluegill	1 ppm	48 hr TLm	(g)
Dimethylamine	Bluegill	166 ppm	48 hr TLm	(g)
Alkanolamine	Chicks	380-765 mg/kg	LD_{50}	(h)
Isopropyl ester	Rats	700 mg/kg	LD_{50}	(h)
Isopropyl ester	Chicks	1420 mg/kg	LD_{50}	(h)
Isopropyl ester	Guinea pigs	550 mg/kg	LD_{50}	(h)
Butyl ester	Rats	620 mg/kg	LD_{50}	(h)
Butyl ester	Guinea pigs	848 mg/kg	LD_{50}	(h)
Butyl ester	Chicks	2000 mg/kg	LD_{50}	(h)
Propylene glycol butyl ether ester	Rats	570 mg/kg	LD_{50}	(h)
Acid	Dogs	100 mg/kg	LD_{50}	(h)
Acid	Chicks	541 mg/kg	LD_{50}	(h)
Unspecified amine	Mallard ducks	2000 mg/kg	LD_{50}	(i)
Acid	Pheasants	472 mg/kg	LD_{50}	(i)
Acid	Mule deer	400-800 mg/kg	LD_{50}	(i)

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ACUTE TOXICITY OF 2,4-D

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Formulation	Organism	Dose
Triethanolamine	Swine	50/mg/kg/day
Butyl ester	Swine	50/mg/kg/day
Triethanolamine	Swine	500 ppm in feed
Triethanolamine	Rats	1000 ppm in water
Triethanolamine	Chicken	1000 ppm in water
Not specified	Dog	500 ppm in feed
Not specified	Rat	1250 ppm in feed
Not specified	Rat	500 ppm in feed

*See summary which follows.

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Duration	Effect	Reference
8-10 doses	Minor transient effects.	(a)
5 doses	None	(a)
1 month	Some locomotory disturbance, depressed growth rate, no gross pathology	(a)
10 mos.	Depressed growth rate, no gross pathology	(a)
Daily from hatching through first 2 mos. of egg production.	Egg size normal, production reduced 30%.	(a)
2 years	None	(b)
2 years	No effects on growth, survival hermatology or tumor incidence.	(b)
2 years	No effects in reproduction studies.	(b)

* CHRONIC TOXICITY OF 2,4-D

Formulation	Organism	Dose	Duration	Effect	Reference
Ethylhexyl ester	Cattle	250/mg/kg/day	14 days	Sick in 3 days, survive & recover from 9 doses. 14 doses lethal.	(c)
Ethylhexyl ester	Sheep	250/mg/kg/day	17 days	Sick in 3 days - 17 doses lethal.	(c)
Ethylhexyl ester	Sheep & Cattle	100/mg/kg/day	10 days	None to minor effects	(c)
2,4-D in water	Human taste threshold	0.01mg/l*		Bad taste	(d)
Alkanolamine	Sheep	100/mg/kg/day	481 days	No effect	(e)
Alkanolamine	Cattle	50/mg/kg/day	112 days	No effect	(e)
Propylene glycol butal ether ester	Sheep	100/mg/kg/day	481 days	No effect	(e)
Alkanolamine	Chicken	100/mg/kg/day	10 days	No effect on weight gain	(f)
Propylene glycol butal ether ester	Chicken	50 mg/kg/day	10 days	No effect on weight gain	(f)
Propylene glycol butal ether ester	Cattle	100mg/kg/day	10 days	No effect	(f)
Acid	Mule deer	80 & 240mg/kg/day	30 days	Minor symptoms - no weight loss	(g)

*mg/l is approximately the same as ppm.

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Chronic Toxicity of 2,4-D

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BY JANICE CROSSLAND AND KEVIN P. SHEA

When buying chemicals, you may get more than you pay for

ONE SOURCE of chemicals potentially toxic to human beings and possibly damaging to the environment is the products of side reactions which occur during the manufacture of agricultural and industrial chemicals. Two recently publicized examples are 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin), a highly poisonous chemical found as a contaminant in the widely used herbicide 2,4,5-T; and the chlorinated dibenzofurans, found as contaminants in some foreign-made polychlorinated biphenyl (PCB) compounds. Both chemicals are far more toxic than the compounds they contaminate and in fact are among the most toxic chemicals known. Thus while attention has been focused on the toxicity of a persistent pesticide, impurities which may in fact be more toxic have gone unnoticed for many years.

Agricultural chemicals, widely dispersed in the environment, usually contain varying amounts of impurities. Recently, workers in the U.S. Department of Agriculture (USDA) analyzed a variety of agricultural chemicals for possible contamination with dioxins. The group of pesticides which were analyzed were all derived, like 2,4,5-T from a group of chemicals known as chlorophenols. One of these chemicals, pentachlorophenol (PCP) turned out to be of particular interest, because it contained significant quantities of the dioxins. Of further interest is the fact that it has been used as a replacement for highly toxic mercury compounds for slime control in the manufacture of paper pulp. Ironically, PCP in paper mill wastewater is turning out to be about as hazardous to aquatic life as are the mercury compounds it is replacing — and more PCP than mercury is needed to counteract the slime.

PCP has been used extensively and for a variety of purposes other than for slime control over the years. As a fungicide it has been used in the processing of oils, leathers, paints, glues, and textiles and has been incorporated into hair shampoos. Application of PCP to wood provides protection against termites and wood-boring insects as well as molds.[1] PCP may also come directly into contact with food — it has been used as a wood preservative in crates used for raw agricultural materials, as a fungicide in beet vats, and in the sackcloth and metal food containers. In 1969 alone 4,000,000 pounds of PCP were produced,[2] and more than one-half of this total was used as wood preservatives.[3] Thus, the possibility of human exposure and of environmental contamination with PCP and its toxic impurities is great.

In the USDA study, 129 samples of 17 different pesticides were analyzed using gas chromatography to identify the presence of the various dioxins.[4] Different dioxins can be distinguished from each other by the number of chlorines which they contain. There are eight positions where chlorine could be attached to the basic dibenzo-dioxin structure, as shown in Figure 1. This makes a variety of dioxins possible ranging from the *mono* form (one chlorine atom) to the *octa* form (eight chlorine atoms). Then, depending on the orientation of these chlorines in space about the molecule, various isomers may also be formed (60 in all are possible for this particular chemical structure. Although the dioxin found in 2,4,5-T is the tetra form (four chlorines are attached to the basic dioxin structure at the 2,3,7, and 8 positions) and this form (TCDD) has received much attention, this is not the only toxic form, as we'll see later.

In the USDA investigation, 23 of 42 samples of 2,4,5-T were contaminated with dioxin as well as 4 of 6 samples of trichlorophenol, all 3 samples of tetrachlorophenol, and 10 of 11 samples of PCP. The dioxins containing 4,6,7, and 8 chlorines were found.

The contamination of PCP by dioxin was confirmed in a later study in which the investigators looked at PCP alone. Both dioxin and another contaminant, dibenzofuran, were found. In all, 17 parts per million (ppm) of the hexachloroxin were found, 105 ppm of the heptachloroxin, and 144 ppm of the octachloroxin. Octa- and heptachlorodibenzofurans were identified, but the quantities were not determined. The basic structure of dibenzofurans is also shown in Figure 1. While the PCP samples used in this study were produced before 1960, the authors pointed out that U.S.

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samples from a 1970 batch in another study also contained dioxins – 0.5 to 37 ppm of the hexa-dioxin and 90 to 135 ppm of the hepta-dioxin. The presence of the dioxins has also been confirmed by studies in other parts of the world – a recent study by Swedish investigators revealed the presence of 50 ppm of octa-dioxin in a sample of PCP.[5]

Formation of Dioxins and Furans

The dioxins found in contaminated samples of 2,4,5-T and in the chlorophenols are formed during the manufacturing process (Figure 1). During the process a reaction between two molecules of trichlorophenol may occur resulting in the formation of tetra-dioxin (TCDD).[6] The formation of TCDD during the manufacture of trichlorophenol and 2,4,5-T has been documented by a number of chemical companies both in the U.S. and Europe.

The same sort of conditions (high temperature, high pressure, and alkalinity) are necessary for the production of PCP when hexachlorobenzene is used as the starting chemical (see reaction 2, Figure 1). Under these conditions both the dioxins and the furans may be formed.[5]

Alternately, the chlorophenols may be produced by the direct condensation of chlorine and phenol (see reaction 3, Figure 1). When too much heat is applied, especially during the manufacture of tetra- and pentachlorophenol, the more highly chlorinated dioxins can be formed.[7]

It has also been proposed that the dioxins may be formed after the manufacture of PCP, for example when wood is treated with this substance under highly alkaline conditions. Wood may also be pressure-impregnated with PCP, with the combination of high pressure and alkalinity leading to the formation of dioxins.

Once in the environment there is the chance that PCP and 2,4,5-T (which may have chlorophenol impurities) can become exposed to very high temperatures. This might occur when brush sprayed with either of these chemicals is burned or when wood products impregnated with PCP are incinerated after disposal. Dioxins might in this way be released into the environment. (One way of producing dioxins, for which there is a Canadian patent, is to heat 5 grams – a gram is equivalent to 0.035 ounce – of PCP at 300 degrees for twelve hours. This yields 1.5 grams of the octa-dioxin.)[8]

Toxicity

Concern about the release of the dioxins into the environment stems from the fact that they are extremely toxic substances. For example, the dose of TCDD needed to kill 50 percent of an experimental group of guinea pigs is 0.6 microgram (one microgram is one-millionth of a gram) per kilogram of body weight.[9] In an experiment in which rabbits were fed TCDD, a dose of 10 micrograms per kilogram killed all the animals, while at a lower dosage of 1 microgram per kilogram, liver damage and chloracne (a skin disease) resulted.[10] PCP on the other hand, is toxic to experimental animals in the milligram per kilogram range.[11] (A milligram is one-thousandth of a gram.)

One of the higher chlorinated dioxins (the hexa-dioxin) found in PCP has also been shown to be toxic. This information was gathered after the accidental poisoning of millions of commercially raised chickens in the U.S. in 1957. In some flocks, there was as high as 50 percent mortality. Affected chickens had droopy and ruffled feathers, as well as difficulty in breathing. Autopsies showed fluid accumulation in the membrane surrounding the heart (hydropericardium), beneath the skin (subcutaneous edema), and in the abdominal cavity, and liver and kidney damage. Until the substance responsible for the epidemic was later identified, it was called the "chick edema factor."[12]

The cause of the disease was at first traced to part of the birds' diet – to the fats added to commercial poultry feed. Later, a toxic fraction was isolated from these fats and found to contain chlorine. It was suggested at first that the substance could be a chlorinated naphthalene.[13] The chlorinated naphthalenes have been known to cause a condition called hyperkeratosis in cattle. (Hyperkeratosis involves thickening and hardening of the skin and proliferation of cells of the mucous membranes.) However, this substance was ruled out when samples of tetrachloronaphthalene and hexachloronaphthalene failed to produce the symptoms of edema disease seen in chickens. Finally, in 1966, X-ray crystallographers at Proctor and Gamble determined that the toxic substance was hexachlordibenzo-p-dioxin.[14] This was confirmed when a chemist at Proctor and Gamble synthesized the hexa-dioxin and showed that it produced chick edema disease.

During the search for the identity of the unknown chemical substance which had caused the disease, samples of toxic fat were tested in a variety of laboratory animals. Adverse effects were found in monkeys, guinea pigs, dogs, rats and pigs.

Because the toxic factor could be recovered from the tissue of chicks fed the substance, the Federal Food and Drug Administration (FDA) ruled in 1961 that commercial fats had to be screened for the chick edema factor before being placed on the market. The chick edema factor had also been found in fat samples which were to be used in food for human beings.[15]

After identifying the factor as a dioxin, the source of the dioxin was still a puzzle. One suggestion was that the dioxin (in contaminated samples of 2,4,5-T or PCP) was originally sprayed onto crops (such as corn) from which vegetable oil was later processed. Another suggestion was that since heat is needed during the preparation of the fats, the dioxins could have been formed as condensation products of PCP or other chlorophenols which might have been present in the original vegetable material.

Furans

The chlorodibenzofurans, which as we mentioned earlier were found as contaminants in PCP samples along with the dioxins are also toxic. Researchers have noted a possible relationship between the toxicity of the PCBs (the polychlorinated biphenyls) and their level of contamination with tetra- and pentachlorodibenzofurans. In a recent study, three samples of PCB-Phenoclor (produced in France), Clophen (produced in Germany) and Aroclor (produced by Monsanto in the U.S.) – were fed to chickens for 60 days, after which the surviving animals were killed and autopsied. The animals receiving Phenoclor and Clophen all died during the course of the experiment; their symptoms included ruffled feathers, loss of feathers, and loss of muscular coordination, and they were found to have hydropericardium, abdominal and subcutaneous edema, and liver necrosis (death of liver cells). On the other hand, only 15 percent of the group receiving Aroclor died during the experiment, and only a few (three of twenty) developed hydropericardium. Five Japanese quail and eight white rats were also fed Phenoclor. The quail all died within 13 days; necrosis of the liver was revealed upon autopsy. All eight rats died within 50 days and showed enlarged livers (with focal necrosis) and disappearance of part of the structure of the spleen.[16]

A subsequent investigation turned up the fact that Phenoclor and Clophen both contained tetra- and pentachlorodibenzofurans. The authors attributed the greater toxicity of these compounds to this factor and suggested that the furans could be formed when crude PCB is distilled.

Occupational Hazard

The chlorinated hydrocarbons have long

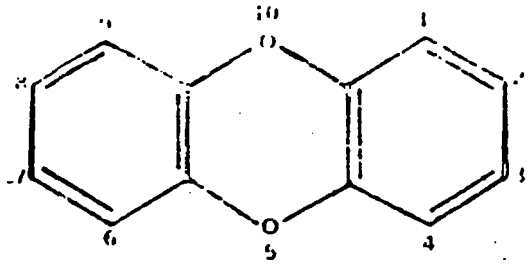
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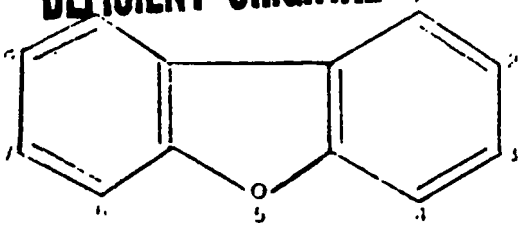
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FIGURE I

FORMATION OF DIOXINS AND FURANS

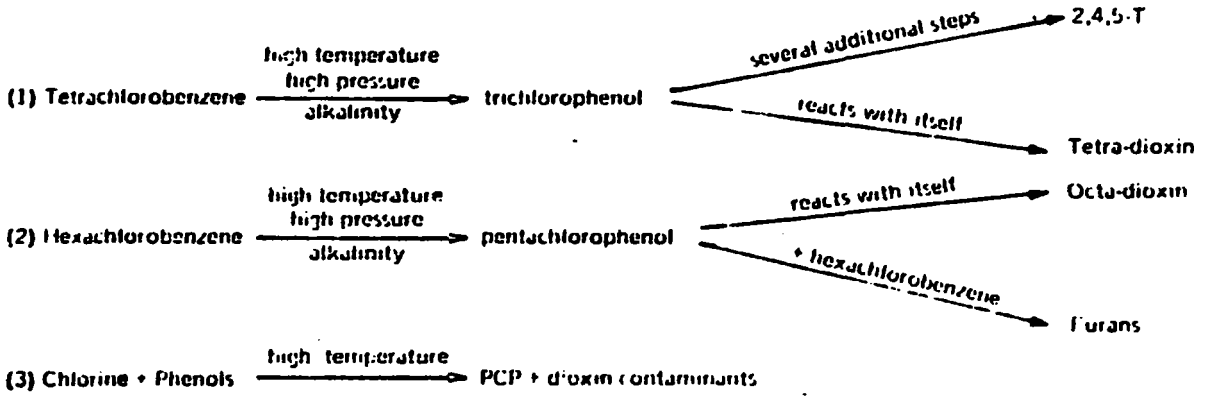


Basic Structure of Dibenzo-dioxin (Dioxin). Chlorine may be attached, in various combinations, at the 1, 2, 3, 4, 5, 6, 7, 8, or 9 positions.



Basic Structure of Dibenzofuran (Furan).

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been a known hazard to workers. The chemicals which we have mentioned so far (PCBs, chlorinated naphthalenes, chlorophenols) cause a condition known as chloracne, one of the most frequently occurring forms of occupational dermatitis (inflammation of the skin).[18] The disease is not caused by chlorine alone, but by chlorine in combination with certain organic substances, such as phenol.

In late 1948, several cases of chloracne were reported in a German chemical plant involved in the production of PCP, using hexachlorobenzene in the process. Some of the symptoms, which the affected men began to show nearly five months after the start of PCP production, included severe acne, in some cases with infections, brown skin pigmentation, and neuralgic (nerve) pain in the lower extremities. It was recognized that either PCP itself or an intermediary product in its production could have been the causative agent. In addition, some of the men had also been exposed to tetrachlorobenzol and trichlorophenol.[19]

A later report again from Germany, in 1958 showed that the contaminants in chlorophenol production indeed can cause chloracne. In this case 81 workers who prepared trichlorophenol and 2,4,5-T developed chloracne. TCDD was isolated from the finished product and was found to be a potent acne-producing agent in rabbits. Tri- and tetrachlorodibenzofuran were also tested on experimental animals and found to be active acne-producing agents.[20] In fact, TCDD proved to be such a strong agent (a 0.05 to 0.001 percent solution was needed to produce acne on the rabbit ear) that in a later attempt to develop a standardized procedure for quantifying the effects of chloracne producing substances TCDD was chosen as the test substance.[21]

Chloracne has also appeared as a problem in U.S. workers. In 1949 an accident in which intermediary chemicals were released at a 2,4,5-T plant (Monsanto) led to 117 cases of chloracne. In a later incident in the U.S., this time at Dow Chemical in 1964, TCDD was singled out as the causative agent of a chloracne outbreak. In order to increase the production rate of 2,4,5-T, Dow had changed its reaction conditions resulting in an increase in TCDD content.[22]

Teratogenicity

Interest in the ability of the dioxin [illegible] produce birth defects began with a study carried out by Bionetics Research Labs between 1965 and 1968 under contract to the National Cancer Institute. 2,4,5-T was among the 48 pesticides which were studied. It was found that pregnant mice, treated with 2,4,5-T at a level of 11.3 milligrams per kilogram of body weight per day produced a greater percentage of abnormal litters of offspring as well as an increase in the percentage of abnormal fetuses per litter. The major type of abnormalities noted were cleft palate and cystic kidney. Abnormalities, as well as increases in fetal mortality, were also produced in rats at various doses. At all doses in the rat, hemorrhage of the gastrointestinal tracts of the fetus were observed. These results, however, could not be definitely linked to 2,4,5-T, because it was discovered that the sample used in the study contained approximately 30 ppm of TCDD.[23]

It has since been learned, however that TCDD alone causes birth defects when fed to pregnant experimental animals. In a study made by Dow Chemical Company, dosage levels [illegible]

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made by workers at the National Institute of Environmental Health Sciences. TCDD was tested on three strains of mice and in one strain of rat. TCDD produced cleft palate in all three strains of mice but not in the rat. Kidney abnormalities were seen in both the mice and the rats.[25]

Environmental Contamination

The dioxins, 2,4,5-T, and PCP may enter the environment from a number of sources. One source of sodium-PCP is the effluent of paper pulp factories which use PCP compounds instead of mercury as slime-controlling agents. Many experiments have shown that PCP, as well as mercury, is toxic to fish.

In one experiment one-half of the guppies tested at a level of 2 ppm PCP were killed in seven days. The guppies could survive a lethal dose of 5 ppm only after first having been acclimated at lower doses (that is, placed in water with a low concentration of PCP for a few days). The authors of this study compared the toxicity of PCP to that of mercury compounds (such as phenylmercuric acetate and mercuric chloride) and found that PCP is as toxic to 25 species of fish as mercury is to guppies, rainbow trout, and stickleback. Furthermore, the amount of PCP needed for efficient slime removal was above the lethal range for many fish, and more PCP than mercury is needed.[26]

The authors noted that the concentration of PCP needed to kill slime was above that which could kill aquatic invertebrates (such as insect larvae and snails). In fact, PCP has been used to kill schistosome-(a type of parasite) carrying snails that live in Egyptian irrigation ditches. Levels of 10 ppm of copper-PCP are lethal to some snails, and 15 to 20 ppm were used on the Egyptian snails. These concentrations, moreover, also affected the local fish population.[27]

While we know that the dioxins are extremely toxic substances, another important question is whether or not they persist in the environment. Although the dioxins are heat stable (TCDD is stable up to 800 degrees centigrade, or 1,472 degrees Fahrenheit) they can be decomposed by sunlight under certain circumstances. Experiments have shown that in alcohol solutions, under natural sunlight, TCDD decomposes rapidly (within hours). When TCDD in methanol was placed on soil (rather than in beakers or closed tubes), however, so that the methanol would evaporate, TCDD had not decomposed after fourteen days. The authors of this study speculated that the waxy cuticle of leaves of a pesticide solvent might serve the same function (chemically) as the soil in that it would protect the dioxin from the ultraviolet rays of the sun. On the other hand, on bare surfaces of soil or water TCDD could accumulate.[28]

A more recent study indicates that accumulation does occur. Three concentrations of TCDD (1, 10, and 100 ppm in benzene) were applied to two types of soil, a sandy soil and a clay loam. Soil samples were then tested after 20, 40, 60, 160, and 350 days. After 350 days, 54 percent of the 1 ppm TCDD sample was recovered for both types of soil. 56 percent of the 100 ppm sample was recovered in the sandy soil, and 71 percent of the 100 ppm sample was recovered in the clay soil.[29]

The possibility that the dioxins could be formed by bacterial action in the soil was also tested, because it has been shown, for example, that one of the breakdown products of propanil (an herbicide), 3,4-dichloroaniline, is converted in the soil to 3,3',4,4'-tetrachloroazobenzene, a substance closely related to chemicals which have produced cancer in experimental animals. Dichlorophenol and trichlorophenol were both incubated in two types of soil in order to determine whether or not a bacterial condensation reaction might convert them to the dioxins. They were incubated at levels of 10, 100, and 1,000 ppm for 70 days — no dioxins, however, were recovered after this time period.

While the chlorophenols and their derivatives represent only a small fraction of the agricultural and industrial chemicals entering the environment, it is clear that they carry with them problems far beyond their own destructive properties. Since almost all organic chemical reactions produce compounds other than the one specifically sought (the popular insecticide Toxaphene was recently found to be composed of 48 different components), the discovery of these toxic contaminants may be a signal that a thorough analysis of widely used chemicals is long over due. ☐

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Chlorinated Dibenzodioxins and Pentachlorophenol

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Introduction

Pentachlorophenol is a registered antimicrobial agent whose principal use is for the preservation of wood. Typical commercial pentachlorophenol contains a variety of substances which are considered to be "inactive" from the aspect of antimicrobial efficacy. Consequently, pentachlorophenol is sold as an antimicrobial agent with 95% active ingredients and 5% "inert" ingredients. Analysis of acceptable commercial pentachlorophenol is shown in Table 1.

The "caustic insolubles," sometimes referred to as the "nonphenolic or neutral impurities," include chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans (*1*). Recently developed analytical technology has allowed quantitation of hexachlorodibenzo-*p*-dioxins and octachlorodibenzo-*p*-dioxin in pentachlorophenol. Portrayed in Table 2 are concentration ranges for these two chlorodibenzo-*p*-dioxins in samples of currently available commercial grade pentachlorophenol. Techniques capable of detecting 0.05 ppm showed no 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in any sample of pentachlorophenol examined by us. The absence of this compound in pentachlorophenol is not surprising, because the appropriate precursors for its formation are not present.

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Table 1. Commercial pentachlorophenol composition.

	Content %
Pentachlorophenol	85–90
Tetrachlorophenol	4–8
Trichlorophenol	<0.1
Higher chlorophenols	2–6
Caustic insolubles (maximum)	1

Table 2. Concentration ranges of some chlorinated dioxins in commercial pentachlorophenol.

Chlorinated dibenzo-*p*-dioxin	Concentration range, ppm
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	None
Hexachlorodibenzo-*p*-dioxins	9–27
Octachlorodibenzo-*p*-dioxin	575–2510

Heptachlorodibenzo-*p*-dioxin and hexa-, hepta- and octachlorodibenzofurans have been qualitatively detected in commercial pentachlorophenol. However, the lack of appropriate standards for these materials does not allow their quantitation.

Severe toxicological responses have been attributed to certain chlorodibenzo-*p*-dioxins (*2*). For example, the LD_{50} of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin ranges from 0.6 μg/kg in male guinea pigs to 115 μg/kg in rabbits of mixed sexes. A benzene solution of this agent containing as little as 0.04 μg/ml produces acne in the rabbit ear bioassay. Very high embryotoxicity and the production of edema in chicks are other properties of this material. The no-effect

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commercial pentachlorophenol. A positive response was noted in both chick edema and rabbit ear bioassays. In the 90-day rat feeding study, untoward effects were noted in a majority of the parameters monitored. Hematological examination revealed a depression of erythrocytes, hemoglobin, and packed cell volumes at a dose level of 30 mg/kg-day pentachlorophenol. Clinical chemistry alterations included an elevation of serum alkaline phosphatase at 30, 10, or 3 mg/kg-day and a depression of serum albumin at 30 or 10 mg/kg-day. The weights of liver and kidneys were increased at 30, 10, or 3 mg/kg-day. Pathologic examination revealed minimal focal hepatocellular degeneration and necrosis at 30 mg/kg-day. Thus, it is evident that commercial pentachlorophenol induced untoward effects in each of the three toxicological tests.

Table 3. Concentrations of chlorinated dioxin "indicators" in commercial pentachlorophenol utilized in toxicological evaluations.

Chlorinated dioxin	Concentration, ppm
Octachlorodibenzo-*p*-dioxin	1980
Hexachlorodibenzo-*p*-dioxins	19

A chemically pure pentachlorophenol having no detectable concentrations of any chlorinated dioxins was subjected to the same toxicological tests. The toxicological data on this chemically pure pentachlorophenol, summarized in Table 5, include negative responses in both the chick edema and rabbit ear bioassays. In the 90-day rat feeding study, the only changes noted were increased liver weights at 30 or 10 mg/kg-day and increased kidney weights at 30 mg/kg-day. However, in contrast to commercial pentachlorophenol, gross and histopathological alterations did not accompany these increases in organ weights. Thus, by utilizing the results of these three tests, it may be concluded that the presence of the contaminants in commercial pentachlorophenol may be detected by toxicological evaluation.

An analysis of a sample of pentachlorophenol representative of that which we are capable of producing is shown in Table 6.

Table 4. Toxicological data on sample of commercial pentachlorophenol.

Study	Result [a]
Chick edema bioassay	+
Rabbit ear bioassay	+
Rat feeding study	
Food consumption	−
Body weight	−
Hematology	
30 mg/kg-day	+
10 mg/kg-day	−
3 mg/kg-day	−
Urinalysis	−
Clinical chemistry	
30 mg/kg-day	+
10 mg/kg-day	+
3 mg/kg-day	+
Liver weight	
30 mg/kg-day	+
10 mg/kg-day	+
3 mg/kg-day	+
Kidney weight	
30 mg/kg-day	+
10 mg/kg-day	+
3 mg/kg-day	+
Pathology	
30 mg/kg-day	+
10 mg/kg-day	−
3 mg/kg-day	−

[a] + denotes effect; − denotes no effect.

The toxicological data on this sample of pentachlorophenol are summarized in Table 7. Both the chick edema and rabbit ear bioassays gave negative responses. In the 90-day rat feeding study, the only unequivocal changes were increased liver weights at 30 or 10 mg/kg-day and increased kidney weights at 30 mg/kg-day. There were no gross or histopathological alterations noted.

To reiterate the toxicological findings on these three samples of pentachlorophenol, a comparison is provided in Table 8.

Commercial pentachlorophenol gave positive responses in both the chick edema and rabbit ear bioassays; in contrast, the chemically pure pentachlorophenol and the improved pentachlorophenol both gave negative responses in these bioassays.

In the rat feeding studies, commercial pentachlorophenol was associated with hema-

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Rodenticide Act. The composition specifications for this new commercial pentachlorophenol are cited in Table 9.

In conclusion, it is feasible to produce a pentachlorophenol in commercial quantities which by comparative evaluations mimics pure pentachlorophenol in toxicological responses.

Table 9. Composition and specifications of improved pentachlorophenol.

	Content
Pentachlorophenol	88–93%
Tetrachlorophenol	12– 7%
Trichlorophenol	<0.1%
Higher chlorophenols	0.1%
Chlorinated dioxins	
Octachlorodibenzo-*p*-dioxin	30 ppm (max.)
Hexachlorodibenzo-*p*-dioxins	1.0 ppm (max.)

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TOXICOLOGY AND APPLIED PHARMACOLOGY 46, 279–303 (1978)

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Results of a Two-Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin in Rats

R. J. KOCIBA, D. G. KEYES, J. E. BEYER, R. M. CARREON, C. E. WADE, D. A. DITTENBER, R. P. KALNINS, L. E. FRAUSON, C. N. PARK, S. D. BARNARD, R. A. HUMMEL, AND C. G. HUMISTON

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Results of a Two-Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin in Rats. KOCIBA, R. J., KEYES, D. G., BEYER, J. E., CARREON, R. M., WADE, C. E., DITTENBER, D. A., KALNINS, R. P., FRAUSON, L. E., PARK, C. N., BARNARD, S. D., HUMMEL, R. A., AND HUMISTON, C. G. (1978). *Toxicol. Appl. Pharmacol.* 46, 279–303. Rats were maintained for 2 years on diets supplying 0.1, 0.01, and 0.001 μg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)/kg/day. Analysis of these diets indicated 2200, 210, and 22 parts per trillion (ppt) of TCDD. Ingestion of 0.1 μg/kg/day caused an increased incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate/nasal turbinates, or tongue, whereas a reduced incidence of tumors of the pituitary, uterus, mammary glands, pancreas, and adrenal gland was noted. Other indications of toxicity at this dose level included increased mortality, decreased weight gain, slight depression of erythroid parameters, increased urinary excretion of porphyrins and δ-aminolevulinic acid, along with increased serum activities of alkaline phosphatase, γ-glutamyl transferase and glutamic–pyruvic transaminase. Gross and histopathologic changes were noted in the hepatic, lymphoid, respiratory, and vascular tissues. The primary hepatic ultrastructural change at this high dose level was proliferation of the rough endoplasmic reticulum. Terminal liver and fat samples from rats at this high dose level contained 24,000 and 8100 ppt of TCDD, respectively. Rats given 0.01 μg/kg/day for 2 years had a lesser degree of toxicity than that seen at the highest dose level. This included increased urinary excretion of porphyrins in females, liver lesions (including hepatocellular nodules), and lung lesions (including focal alveolar hyperplasia). Terminal liver and fat samples from rats of this dose level contained 5100 and 1700 ppt of TCDD, respectively. Ingestion of 0.001 μg of TCDD/kg/day (~22 ppt in the diet) caused no effects considered to be of any toxicologic significance. At this lower dose level, terminal liver and fat samples each contained 540 ppt of TCDD. These data indicate that continuous doses of TCDD sufficient to induce severe toxicity increased the incidence of some types of tumors, while reducing other types. During the 2-year study in rats, no increase in tumors occurred in those rats receiving sufficient TCDD to induce slight or no manifestations of toxicity.

The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a highly toxic impurity that may be formed under certain conditions during the production of 2,4,5-trichlorophenol. TCDD has been considered one of the causes of chloracne, which has been

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associated with the industrial production of 2,4,5-trichlorophenol and other products made from 2.4.5-trichlorophenol.

Most of the earlier toxicologic studies with TCDD were concerned with the assessment of its short-term toxicity and teratogenic potential. Results of these earlier studies have been summarized in a previous publication by Kociba *et al.* (1976). This same publication also reported the results of a subchronic study in which rats were given 1.0, 0.1, 0.01, 0.001, or 0 μg of TCDD/kg 5 days/week for 13 weeks. Doses of 1.0 μg/kg/day caused multiple toxicologic effects, including mortality and morphologic changes in liver, thymus, and reproductive organs. A dose level of 0.1 μg/kg/day caused lesser degrees of toxicity, and rats given 0.01 or 0.001 μg/kg/day had no alterations considered of any toxicologic significance.

More recently, toxicologic studies of TCDD have also been conducted in the monkey. McConnell *et al.* (1978) reported a single oral LD50 in monkeys of <70 μg TCDD/kg. These monkeys died with loss of hair or nails, keratinization of Meibomian glands and hair follicles, and hyperplasia of the epithelium of the renal pelvis, stomach, and bile duct.

Allen *et al.* (1977) reported on a subchronic study in which monkeys consumed a diet containing 500 ppt of TCDD for 9 months. It was calculated that these monkeys ingested 2 to 3 μg TCDD/kg over the course of the 9-month study. Clinically, these monkeys showed changes similar to those described by McConnell *et al.* (1978) as well as hematologic depression and hemorrhages in various tissues. Hypertrophy, hyperplasia, and/or metaplasia were noted in the epithelium of the bile ducts, salivary glands, bronchi, pancreatic ducts, sebaceous glands, skin, gastric lining, and urinary tract.

In regard to long-term or carcinogenic studies conducted in rodents with TCDD, Innes *et al.* (1969) reported no increase in tumors in mice given 2,4,5-Trichlorophenoxyacetic acid contaminated with a level of TCDD sufficient to supply 0.27 μg of TCDD/kg/day. DiGiovanni *et al.* (1977) conducted a study in which TCDD was reported to be only a weak tumor initiator in the two-stage system of mouse skin carcinogenesis with 7,12-dimethylbenz[a]anthracene (DMBA).

Van Miller and Allen (1977) issued a preliminary report on a study of small groups of male rats fed diets containing TCDD for 65 weeks. All 10 rats of each group receiving 1.0, 0.5, or 0.05 ppm of TCDD in the diet died within 4 weeks, with acute toxic effects, including severe liver necrosis, bile duct hyperplasia and edema, atrophy of spleen and thymus, gastrointestinal hemorrhages, and decreased spermatogenesis. Groups of male rats on diets containing 5000 or 1000 ppt experienced increased mortality, decreased weight gain, and liver toxicity. Dietary levels of 500, 50, 5, and 1 ppt of TCDD were also studied. Various neoplasms were found in some rats at all dose levels of 5 ppt and higher, with only the lowest dose level of 1 ppt reportedly free of any neoplasms. These same results on tumorigenesis were included in an updated report by Van Miller *et al.* (1977), which included the data generated through the end of their 95-week study. No tumors were reported in the group given 1 ppt of TCDD or in a total of 50 control rats.

In view of the need for an evaluation of the chronic toxicity and potential for carcinogenicity of TCDD, the study reported herein was conducted. In this study, groups of male and female rats were maintained for 2 years on diets supplying

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various dose levels of TCDD, and numerous parameters were evaluated in order to assess the potential chronic toxicity associated with long-term ingestion of the material.

METHODS

Experimental design. Male and female Sprague–Dawley rats, Spartan substrain,[1] 6 to 7 weeks old, were randomly placed (two/cage) into suspended wire-bottomed cages for this study. Food[2] and water were available *ad libitum*. Groups of 100 rats (50 males, 50 females) were maintained for up to 2 years on diets supplying 0.1, 0.01, or 0.001 μg of TCDD/kg/day. The diet of the control group of 172 rats (86 males, 86 females) contained the vehicle.

Test material. The TCDD sample used for this 2-year study was prepared by the Dow Chemical Company. Purification of the crude TCDD was followed by gas chromatography and mass spectrometry. The final product had a purity exceeding 99%, as determined by electron-capture gas chromatography. This sample was used to prepare the premixes and test diets according to the following general procedure: Approximately 1 mg of TCDD was weighed on a microbalance and dissolved in 40 ml of reagent-grade acetone. This solution was then added to 1000 g of control feed and mixed for 30 min to prepare a stock premix. A sufficient sample of the stock premix was then mixed with control feed to produce a working premix to be used in preparing the test diets. The stock premix was prepared six times during the course of the study. The test diets were prepared by diluting the working premix with sufficient control feed to provide dose levels of TCDD as required by body weight and food consumption determinations in order to maintain the designated dosages on a microgram per kilogram per day basis. Samples of the working premix and the test diets were analyzed periodically to ascertain that the dietary levels were being maintained as scheduled. The concentration of TCDD in the premix samples was determined using electron-capture gas chromatography and gas chromatography–mass spectrometry. The concentration of TCDD in the test diets was determined by gas chromatography-mass spectrometry after extraction and suitable cleanup.

Clinical observations. All rats were palpated on a monthly basis, with a recording of the number of rats bearing palpable masses. Body weights and food consumption of 20 rats/sex/treatment level and controls were routinely recorded for each week of the first 3 months of the study and at approximately monthly intervals thereafter. All remaining rats were weighed monthly throughout the study.

Blood samples for hematological determinations were collected from eight rats/sex/group at 3, 12, and 23 months of treatment. The total erythrocyte count (RBC), total and differential leukocyte counts (WBC), thrombocyte and reticulocyte counts, packed cell volume (PCV), and hemoglobin (Hgb) concentration were determined using automated[3] or manual procedures. Urine samples were collected from seven to eight rats/sex/group at these same time intervals. Urine specific gravity, pH, and the presence

[1] Spartan Research Animals, Haslett, Michigan.
[2] Purina Laboratory Chow, Ralston-Purina Co., St. Louis, Missouri.
[3] Coulter Counter Model ZB-1. Coulter Electronics, Hialeah, Florida.

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or absence of sugar, protein, ketones, bilirubin, and occult blood were determined[4] at each of these times, and urinary urobilinogen was also evaluated at Month 23.

Urinary excretion of creatinine, coproporphyrin, uroporphyrin, and δ-amino levulinic acid (δ-ALA) was determined by a consulting laboratory[5] on samples collected from four to five rats/sex/group at Months 3–4, 12, and 23.

Serum samples were collected from seven rats/sex/group by orbital puncture at Month 22 for determination of urea nitrogen (BUN), glutamic pyruvic transaminase (SGPT), bilirubin (total, direct and indirect), cholesterol, and triglycerides. Automated procedures were used for these determinations.[6] Serum samples were similarly collected from seven rats/sex/group at Month 23 for determination of alkaline phosphatase (AP) activity, total protein, albumin and globulin.[6]

At terminal necropsy, serum samples were collected from all survivors or a maximum of 10 rats/sex/group for determination of BUN, SGPT, AP, and total bilirubin.[6] A consulting laboratory[5] also made determinations of serum γ-glutamyl transferase activity (GGT).

All rats dying or culled during the course of the study were subjected to a gross pathologic examination. Representative portions of the major organs and any gross lesion suggestive of a significant pathologic process or tumor formation were collected from each rat and preserved in buffered 10% formalin.

Terminal necropsy examination was conducted at the end of 2 years of treatment (105th week). All rats were deprived of food overnight prior to killing by decapitation. The eyes of all rats were examined by gently pressing a glass slide against the cornea under bright fluorescent illumination. Any observations on the eyes were recorded as part of the gross necropsy observation records. The eyes for a maximum of five rats/sex/group were preserved in Zenker's fixative. Eyes from remaining rats were fixed in formalin fixative.

The weights of the liver, kidney, brain, heart, thymus, spleen, testes, and ovaries/uterus were recorded for a maximum of 10 rats/sex/group. A bone marrow smear was prepared from most rats, and filed for future reference, if indicated. Portions of fat, liver, and kidney from a maximum of five rats/sex/group were frozen for possible TCDD analysis, with subsequent analysis of liver and fat samples from three females/dose level, using gas chromatography–low-resolution mass spectrometry. Portions of esophagus, salivary glands, stomach, small intestine, large intestine, pancreas, liver, kidneys, urinary bladder, prostate, accessory sex glands, epididymis, testes, ovaries, uterus, brain (cerebrum, cerebellum, brain stem), pituitary gland, spinal cord, peripheral (sciatic) nerve, trachea, lungs, spleen, thymus, lymph nodes, heart, aorta, skeletal muscle, mammary tissue (females), adrenal glands, thyroid, parathyroid, tongue, lower jaw, and skull (including nasal turbinates, ear canal), together with any additional gross lesions were preserved in formalin fixative.

Histologic examination of tissues was conducted on paraffin-embedded sections of tissues which were stained with hematoxylin and eosin. All rats from the control and top dose level, regardless of whether they died during the study or were killed at the

[4] Ames Bililabstix or Multistix, Ames Co., Elkhart, Indiana and TS Meter, AO Optical, Buffalo, New York.

[5] Bioscience Laboratories, Van Nuys, California.

[6] Technicon AutoAnalyzer, Technicon Corp., Rye, New York.

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termination, were subjected to histologic examination of an extensive list of tissues, intended to include a majority of those tissues listed above as those collected at the time of terminal necropsy. All rats from the two lower dose levels were subjected to histologic examination of those selected tissues identified as possible target organs and all gross lesions suggestive of tumor formation. The actual number of tissues specimens from each group examined histologically is on file and available from the authors. Additional sections of liver from selected females from the terminal necropsy were stained for lipid content using Oil Red O or Sudan IV stains.

Liver tissue collected at the terminal necropsy was examined using an electron microscope[7] to characterize qualitatively the ultrastructure of hepatocytes from three females/group. The liver tissue was fixed in 2.5% phosphate-buffered glutaraldehyde and then postfixed in 1% phosphate-buffered osmium tetroxide, dehydrated through graded ethanol solutions, washed in propylene oxide, infiltrated with Epon 812, and embedded in polyethene capsules. Sections of 1 μm thickness were stained with toluidine blue and examined using light microscopy. Thin sections were stained with uranyl acetate and lead citrate prior to examination by electron microscopy.

Statistical evaluation of data. The significance of differences between control and test values for hematology, urinary and clinical chemistry parameters, body weights, organ weights, and organ/body weight ratios was statistically determined by one-way analyses of variance followed by the Dunnett test (Steel and Torrie, 1960). A significance level of $p < 0.05$ was used. Data on mortality, palpable masses, gross pathology, histopathology, and tumor incidences were analyzed using the Fischer exact probability test, $p < 0.05$, one-sided test (Siegel, 1956). Mortality data were also analyzed using the Mantel–Haenszel Test. Repeated measures analyses across time were not appropriate because of mortality and because the assumptions of the statistical tests were not valid.

Statistical evaluation of gross pathology data collated for the entire study compared the data of each of the treatment groups with the data of the control group of that sex. Statistical evaluation of histopathologic observations and tumor incidences compared the data of the high-dose group with the data of the control group of that sex. The same evaluation was conducted on the lower dose levels in instances in which comparable numbers of tissues were subjected to microscopic examination (apparent target organs).

RESULTS

Dietary Content of TCDD

Analyses of feed samples indicated the dosage levels of 0.1, 0.01, and 0.001 μg of TCDD/kg/day equated with approximately 2193, 208, and 22 ppt of TCDD in the diet. Six repeated analyses of the feed samples indicated good agreement between the intended content of TCDD and results of analysis for TCDD content.

Clinical observations

Females given 0.1 μg/kg/day had statistically increased cumulative mortality during the latter half of the study, whereas those given 0.01 or 0.001 μg/kg/day had mortality

[7] Carl Zeiss, Inc., New York.

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rates comparable to that of the controls. In males, there were some isolated instances of statistical differences between the treated and control groups. However, as the mortality of only the group of males given 0.01 μg/kg/day was significantly different from control using the Mantel–Haenszel test, these deviations in the male rats were considered of questionable toxicologic significance. Mean body weights of males and females given 0.1 μg/kg/day were statistically decreased from control values throughout the major portion of the study, from Month 6 to the end of the 2-year test period. Mean body weights of females given 0.01 μg/kg/day were decreased to a lesser degree during this same time interval. The mean body weights of males given 0.01 or 0.001 μg/kg/day and females given 0.001 μg/kg/day were sometimes lower than controls during the middle of the study, but only occasionally were the differences statistically significant. During the last quarter of the study body weights of these groups were comparable to those of controls.

There were no consistent deviations in food consumption of males or females at any dose. The few sporadic cases in which there was a statistical increase or decrease between the control and treatment groups followed no consistent trend, and were considered of no toxicologic significance. The first palpable mass was noted at Month 5 in a male of the control group. There were no statistically significant differences between the control and treated groups except during Months 15 and 16, when the males given 0.01 μg/kg/day had an increased incidence of palpable masses. This was considered of no toxicologic significance because of its isolated occurrence and lack of dose response. During the last 12 months of the study, females ingesting 0.1 μg/kg/day had a consistent trend toward a decrease in the number of rats with palpable masses. This observation was not noted at lower dosage levels in the females.

Hematology

The hematology data collected after 23 months of treatment are listed in Table 1 and are similar to the patterns observed during the study. In rats given 0.1 μg/kg/day, there were statistically significant decreases in the PCV and Hgb values for males after 3 months as well as decreases in the Hgb values for males and decreases in PCV, total RBC, and WBC counts and Hgb values for females after 1 year. At the preterminal examination, this high dose group again had statistically significant decreases in RBC and Hgb values (males) and PCV and Hgb values (females); reticulocyte counts also appeared to be slightly increased. Thrombocyte and WBC differential counts appeared to be unaffected at all dose levels of TCDD. Rats given 0.01 or 0.001 μg/kg/day had no hematologic changes considered related to treatment.

Urinalyses

Repeated examination of urinary parameters revealed no consistent alterations that could be attributed to any of the dose levels of TCDD.

Urinary porphyrins and δ-ALA

Porphyrin data collected after 23 months of treatment are listed in Table 2 and are representative of the patterns observed during the study. Urinary excretion of coproporphyrin was statistically increased in female rats at a dose level of 0.1 μg/kg/day after each evaluation at 3–4, 12, and 23 months. Coproporphyrin excretion was also

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TABLE 1

MEAN HEMATOLOGIC VALUES OF MALE RATS (DAY 681) AND FEMALE RATS (DAY 682) ON DIETS CONTAINING TCDD

Dose of TCDD (μg/kg/day)	Sex	Number of rats/group	PCV (%)	RBC (×10⁶/mm³)	Hgb (g/100 ml)	Reticulocytes (%)	Thrombocytes (×10⁶/mm³)	WBC (×10³/mm³)	WBC differential count (%)				
									Neut	Lymph	Mono	Eosin	Baso
0	M	8	46.9 ± 4.7[a]	7.99 ± 0.64	15.6 ± 1.6	1.0	1.195 ± 0.350	14.9 ± 5.4	27	65	7	1	0
0.100	M	8	43.4 ± 4.3	7.19 ± 0.65[b]	13.9 ± 1.5[b]	2.2	1.214 ± 0.358	12.1 ± 5.2	33	62	4	1	0
0.010	M	8	47.6 ± 2.8	7.65 ± 0.46	16.0 ± 0.8	1.0	1.482 ± 0.342	15.8 ± 4.0	34	60	5	1	0
0.001	M	8	47.4 ± 1.8	7.73 ± 0.27	15.7 ± 0.6	0.5	1.042 ± 0.143	18.7 ± 8.0	30	65	4	1	0
0	F	8	43.6 ± 1.4	6.84 ± 0.66	14.1 ± 1.1	0.8	1.046 ± 0.210	9.1 ± 1.5	34	60	4	2	0
0.100	F	8	38.9 ± 3.8[b]	6.38 ± 0.90	12.5 ± 1.2[b]	1.2	1.176 ± 0.358	7.1 ± 2.1	30	67	2	1	0
0.010	F	8	45.1 ± 1.1	7.35 ± 0.32	15.1 ± 0.4	0.5	0.962 ± 0.161	9.4 ± 2.2	36	60	3	1	0
0.001	F	8	46.9 ± 4.7	7.56 ± 0.69	15.7 ± 1.5[b]	0.5	0.857 ± 0.239	9.5 ± 1.9	23	72	3	2	0

[a] Mean ± SD.
[b] Statistically significant from control mean using analysis of variance and Dunnett's test, $p < 0.05$.

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TABLE 2

URINARY EXCRETION OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN, AND δ-AMINO-LEVULINIC ACID FOR MALE AND FEMALE RATS (DAYS 678–680) ON DIETS CONTAINING TCDD

Dose TCDD (μg/kg/day)	Sex	Number of rats/group	Total urine vol, 48 hr (ml)	Creatinine (mg/48 hr)	Coproporphyrin (μg/48 hr)	μg Coproporphyrin / mg Creatinine	Uroporphyrin (μg/48 hr)	μg Uroporphyrin / mg Creatinine	δ-amino-levulinic acid (mg/48 hr)	mg δ-ALA / mg Creatinine
0	M	4	51.0 ± 21.6[a]	27.6 ± 3.2	18.0 ± 3.7	0.69 ± 0.19	5.4 ± 2.2	0.200 ± 0.092	0.27 ± 0.38	0.010 ± 0.015
0.100	M	5	63.4 ± 40.5	19.6 ± 9.1	19.6 ± 11.7	1.22 ± 1.08	7.3 ± 4.0	0.418 ± 0.295	0.08 ± 0.02	0.005 ± 0.003
0.010	M	5	61.4 ± 26.1	24.4 ± 5.7	16.5 ± 8.0	0.64 ± 0.25	5.7 ± 2.1	0.228 ± 0.056	0.26 ± 0.42	0.009 ± 0.013
0.001	M	5	41.0 ± 6.4	30.8 ± 3.2	23.8 ± 4.8	0.78 ± 0.19	5.3 ± 1.6	0.174 ± 0.053	0.06 ± 0.01	0.002 ± 0.001
0	F	5	60.0 ± 34.9	23.3 ± 6.2	9.8 ± 1.3	0.43 ± 0.49	3.8 ± 1.7	0.157 ± 0.050	0.07 ± 0.03	0.003 ± 0.001
0.100	F	5	51.2 ± 22.8	18.6 ± 4.3	17.4 ± 4.0[b]	0.98 ± 0.41[b]	5.7 ± 2.3	0.296 ± 0.074[b]	0.12 ± 0.05	0.006 ± 0.002[b]
0.010	F	5	54.2 ± 20.5	19.4 ± 2.3	16.4 ± 4.7[b]	0.83 ± 0.18	3.5 ± 1.1	0.181 ± 0.053	0.08 ± 0.03	0.004 ± 0.002
0.001	F	5	57.2 ± 20.0	20.8 ± 4.7	8.6 ± 2.0	0.42 ± 0.06	3.0 ± 1.1	0.143 ± 0.037	0.08 ± 0.02	0.004 ± 0.001

[a] Mean ± SD.

[b] Statistically significant from control mean by analysis of variance and Dunnett's test, $p < 0.05$.

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statistically increased in female rats at a dose level of 0.01 μg/kg/day after 3 and 23 months. Urinary excretion of uroporphyrin was statistically increased in females after 3 and 23 months of receiving 0.1 μg/kg/day and after 3 months of receiving 0.01 μg/kg/day. Urinary excretion of δ-ALA was statistically increased in females after 3 and 23 months of receiving 0.1 μg/kg/day. Total urine volume or creatinine excretion was not affected by any of these dose levels in the females. Males had no alterations considered treatment-related in any of these parameters at any of the dose levels of TCDD.

Clinical Chemistry

For sake of brevity, only the results obtained at terminal necropsy after 2 years of treatment are presented in Table 3. Analyses of serum samples collected by orbital

TABLE 3

MEAN TERMINAL (2-YEAR) CLINICAL CHEMISTRY VALUES FOR MALE AND FEMALE RATS GIVEN DIETS CONTAINING TCDD

Dose TCDD (μg/kg/day)	Sex	Number of rats/group	BUN (mg/100 ml)	SGPT (mU/ml)	AP (mU/ml)	Total bilirubin (mg/100 ml)	γ-glutamyl transferase (mU/ml)
0	M	10	33 ± 34[a]	49 ± 17	87 ± 30	0.2 ± 0	0 ± 0
0.100	M	5	28 ± 11	42 ± 6	105 ± 17	0.2 ± 0	1 ± 0
0.010	M	4	36 ± 19	47 ± 10	88 ± 26	0.2 ± 0	1 ± 0
0.001	M	10	20 ± 9	43 ± 9	86 ± 25	0.2 ± 0	0 ± 0
0	F	10	21 ± 9	39 ± 11	60 ± 29	0.2 ± 0	0 ± 0
0.100	F	4	20 ± 3	54 ± 12[b]	205 ± 146[b]	0.2 ± 0	14 ± 10[b]
0.010	F	10	17 ± 3	49 ± 7	61 ± 20	0.3 ± 0.1	1 ± 0
0.001	F	10	18 ± 5	42 ± 5	54 ± 28	0.4 ± 0.2	1 ± 0

[a] Mean ± SD.
[b] Statistically significant from control mean using analysis of variance and the Dunnett's test. $p < 0.05$.

puncture at 22 to 23 months of treatment revealed no alterations considered related to treatment in regard to BUN, SGPT, total, direct, or indirect bilirubin, cholesterol, triglycerides, total protein, albumin, and globulin. Serum AP was statistically increased in females given 0.1 μg/kg/day. A statistically significant increase in serum triglycerides noted in males given 0.01 μg/kg/day was considered of no toxicologic significance based on the lack of a dose-response relationship. Analyses of serum samples collected at terminal necropsy after 2 years indicated a statistical increase in SGPT, AP, and GGT activities for females given 0.1 μg/kg/day. Females at the lower dose levels and males at all dose levels were unaffected in these parameters. The BUN and total bilirubin values of either sex were unaffected by any level of treatment with TCDD.

Gross and Microscopic Observations on Tissues

Detailed descriptions of all gross and microscopic observations made on all rats killed or dying during the course of the 2-year study are on file and available from the authors. On account of the voluminous nature of the data, the results are summarized below. Tumor and tumor-like lesions are listed in Tables 4 and 5.

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TABLE 4

TUMOR INCIDENCE IN MALE RATS MAINTAINED ON DIETS CONTAINING TCDD[a]

Time intervals during study:	Months 13–24				Terminal kill				Total			
Dose level in μg/kg/day:	0	0.1	0.01	0.001	0	0.1	0.01	0.001	0	0.1	0.01	0.001
Number of rats examined:	65	41	46	38	15	5	4	11	85	50	50	50
Rats with tumors/tumor like lesions:												
Hepatocellular hyperplastic nodule(s)	2	1	2	0	4	1	1	0	6	2	3	0
Hepatocellular carcinoma(s)	1	0	0	0	1	1	0	0	2	1	0	0
Bile duct adenoma	0	1	0	0	0	0	0	0	0	1	0	0
Stratified squamous cell carcinoma of hard palate or nasal turbinates	0	4	0	0	0	0	0	0	0	4[b]	0	0
Paravertebral or sub cutaneous malignant schwannoma	0	1	1	0	0	0	0	0	0	1	1	0
Carcinoma of renal tubules pelvis, or bladder	1	0	0	0	1	0	0	1	2	0	0	1
Adenoma of renal tubules or pelvis	0	0	0	1	0	0	0	1	0	0	0	2
Keratinizing squamous cell carcinoma of lung	0	1	0	0	0	0	0	0	0	1	0	0
Pulmonary adenoma	1	1	0	0	0	0	0	0	1	1	0	0
Pulmonary adenocarcinoma	1	0	0	0	0	0	0	0	1	0	0	0
Oligodendroglioma/astro-cytoma of brain, or glioma of spinal cord	2	0	0	1	0	0	0	0	2	0	0	1
Interstitial cell adenoma of testes	2	0	0	1	0	0	0	1	2	0	0	2
Adenoma of prostate	0	0	0	0	0	0	0	1	0	0	0	1
Subcutaneous fibroadenoma/ fibroma/lipoma	8	6	4	1	1	0	1	0	10	6	5	1[b]

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Subcutaneous fibrosarcoma	0	1	1
Benign mammary neoplasms	0	1	0
Mammary gland adeno-carcinoma	2	0	0
Cutaneous papilloma/basal cell tumor	3	0	0
Squamous cell carcinoma of integument	0	0	0
Zymbal gland carcinoma	0	0	1
Pituitary adenoma	19	10	11
Pituitary adenocarcinoma	1	0	0
Stratified squamous cell carcinoma of tongue	0	3	1
Fibrosarcoma of tongue	1	0	0
Squamous papilloma/polyp of gastric mucosa	0	1	1
Mucocystadenocarcinoma of small intestine	1	0	0
Leiomyosarcoma of cecum	0	0	0
Intraabdominal schwannoma/sarcoma	0	2	0
Acinar adenoma of pancreas	11	2	4
Acinar adenocarcinoma of pancreas	1	0	0
Islet adenoma of pancreas	6	3	2
Islet adenocarcinoma of pancreas	1	0	0
Adenoma of adrenal cortex	0	5	2
Pheochromocytoma of adrenal	19	3	10
Interfollicular C-cell adenoma of thyroid	2	4	0
Interfollicular C-cell adenocarcinoma of thyroid	2	1	0
Follicular adenoma of thyroid	0	1	1
Follicular adenocarcinoma of thyroid	0	0	0

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0	0	0	0	0	0	1	1	0
0	0	0	0	0	0	1	0	0
0	0	0	0	0	2	0	0	0
0	0	0	0	0	3	0	0	0
1	0	0	0	0	0	0	0	1
1	0	0	0	0	0	0	1	1
5	7	2	0	1	26	13	11	6
0	1	0	0	0	3	0	0	0
1	0	0	0	0	0	3[b]	1	1
0	0	0	0	0	1	0	0	0
1	0	0	0	0	0	1	1	1
2	0	0	0	0	1	0	0	2
0	0	1	0	0	0	1	0	0
0	0	0	0	0	0	2	0	0
3	3	0	1	4	14	2[b]	5	7
0	0	0	0	0	1	0	0	0
1	6	0	1	2	12	3	3	3
0	1	0	0	0	2	0	0	0
0	0	0	0	0	0	5[b]	2	0
3	9	1	0	3	28	4[b]	10	6
0	6	0	0	0	8	4	0	0
0	1	0	0	0	3	1	0	0
0	0	0	0	0	0	1	1	0
0	0	1	0	0	0	1	0	0

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TABLE 4—*continued*

Time intervals during study:	Months 13–24				Terminal kill				Total			
Dose level in μg/kg/day:	0	0.1	0.01	0.001	0	0.1	0.01	0.001	0	0.1	0.01	0.001
Number of rats examined:	65	41	46	38	15	5	4	11	85	50	50	50
Malignant lymphoreticular neoplasm	5	0	3	2	0	0	0	1	5	0	3	3
Hemangioma of lymph node	1	0	0	0	0	0	0	0	1	0	0	0
Fibrosarcoma/osteosarcoma of musculoskeletal system	1	0	0	0	0	0	0	0	2	0	0	0
Intraorbital malignant schwannoma	0	0	1	0	0	0	0	0	0	0	1	0
Mediastinal fibrosarcoma	0	0	0	1	0	0	0	0	0	0	0	1
Not available for pathological examination	0	0	0	0	0	0	0	0	1	0	0	0

[a] No tumors occurred during Months 1 through 6. Tumors occurring during Months 7 to 12 included 1 subcutaneous fibroadenoma (control), 1 pituitary adenoma (0.1 μg/kg/day), 1 pituitary adenocarcinoma (control), 1 osteosarcoma (control). These four tumors, which were present in the 10 males dying prior to Month 13, are included in the above total tabulation.

[b] Statistically different from control data when analyzed using the Fischer exact probability test, $p < 0.05$. Appropriate tumor data have been combined for the sake of brevity.

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TABLE 5

TUMOR INCIDENCE IN FEMALE RATS MAINTAINED ON DIETS CONTAINING TCDD[a]

Time intervals during study:	Months 13–24				Terminal kill				Total			
Dose level in μg/kg/day:	0	0.1	0.01	0.001	0	0.1	0.01	0.001	0	0.1	0.01	0.001
Number of rats examined:	60	36	34	32	25	4	14	16	86	49	50	50
Rats with tumors/tumor-like lesions:												
Hepatocellular hyperplastic nodules	2	20	8	1	6	3	10	2	8	23[b]	18[b]	3
Hepatocellular carcinoma(s)	0	10	1	0	1	1	1	0	1	11[b]	2	0
Bile duct adenoma	0	2	0	0	0	0	0	1	0	2	0	1
Stratified squamous cell carcinoma of hard palate or nasal turbinates	0	4	1	0	0	0	0	0	0	4[b]	1	0
Keratinizing squamous cell carcinoma of lung	0	7	0	0	0	0	0	0	0	7[b]	0	0
Pulmonary adenocarcinoma	0	0	1	0	0	0	0	0	0	0	1	0
Astrocytoma of cerebrum	0	0	0	0	1	0	0	0	1	0	0	0
Malignant schwannoma of pelvic canal	1	0	0	0	0	0	0	0	1	0	0	0
Nephroblastoma of kidney	0	1	1	0	0	0	0	0	0	1	1	0
Adenoma of renal tubules	0	0	0	1	0	0	0	0	0	0	0	1
Carcinoma of renal pelvis	0	0	0	0	0	0	1	0	0	0	1	0
Granulosal cell neoplasm of ovary	0	0	1	1	3	0	0	0	3	0	1	1
Benign tumor of uterus	16	3	7	5	12	2	4	7	28	7[b]	11	12
Malignant schwannoma of uterus	2	0	3	1	0	0	0	0	2	0	3	1
Adenocarcinoma of uterus	6	4	0	1	0	0	0	0	6	4	0	1
Fibroma of cervix/vagina	1	0	0	0	1	0	1	0	2	0	1	0
Subcutaneous fibroma/ fibrolipoma	1	0	0	1	0	0	0	0	1	0	0	1

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TABLE 5—*continued*

Time intervals during study:	Months 13–24				Terminal kill				Total			
Dose level in μg/kg/day:	0	0.1	0.01	0.001	0	0.1	0.01	0.001	0	0.1	0.01	0.001
Number of rats examined:	60	36	34	32	25	4	14	16	86	49	50	50
Subcutaneous fibrosarcoma	0	0	0	1	0	0	0	0	0	0	0	1
Benign neoplasm of mammary gland	50	22	23	24	23	2	12	11	73	24[b]	36	35
Carcinoma of mammary gland	5	0	1	3	6	0	5	1	8	0[b]	4	4
Stratified squamous cell carcinoma of digit	0	1	0	0	0	0	0	0	0	1	0	0
Cystadenoma of Zymbal gland	0	0	0	0	0	0	0	1	0	0	0	1
Pituitary adenoma	26	12	8	12	17	0	5	6	43	12[b]	13	18
Pituitary adenocarcinoma	4	2	0	0	2	0	1	0	6	2	1	0
Stratified squamous cell carcinoma of tongue	1	2	0	0	0	0	0	0	1	2	0	0
Papilloma of esophagus	0	0	0	0	1	0	0	0	1	0	0	0
Squamous papilloma/polyp of gastric mucosa	0	2	0	0	1	0	0	1	1	2	0	1
Leiomyosarcoma/sarcoma of small intestine	1	0	0	0	0	0	1	0	1	0	1	0

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Polypoid adenoma of large intestine	1	0	0	0	0	0	0	0	1	0	0	0
Acinar adenoma of pancreas	0	1	0	0	0	0	0	1	0	1	0	1
Islet cell adenoma of pancreas	1	0	0	1	2	0	1	2	3	0	1	3
Islet cell adenocarcinoma of pancreas	1	0	0	0	1	0	0	0	2	0	0	0
Adenoma of adrenal cortex	7	5	1	2	2	0	1	4	9	5	2	6
Pheochromocytoma of adrenal	4	3	0	1	3	0	1	1	7	3	1	2
Interfollicular adenoma of thyroid	9	4	1	1	5	0	1	0	14	4	2	1
Interfollicular adeno-carcinoma of thyroid	3	2	0	1	1	0	0	1	4	2	0	2
Follicular adenoma of thyroid	0	1	0	0	0	0	0	0	0	1	0	0
Malignant lymphoreticular neoplasm	1	1	1	0	1	0	0	0	2	1	1	0
Hemangioma of abdominal muscle	1	0	0	0	0	0	0	0	1	0	0	0
Not available for pathologic examination	0	0	0	0	0	0	0	0	0	1	0	0

[a] No tumors occurred during Months 1 through 6. Tumors occurring during Months 7 to 12 included two benign tumors of the uterus (0.1 μg/kg/day) and one benign neoplasm of the mammary gland (0.01 μg/kg/day). These three tumors, which were present in the 14 females dying prior to Month 13, are included in the above total tabulation.

[b] Statistically different from control data when analyzed using the Fischer exact probability test, $p < 0.05$. Appropriate tumor data have been combined for the sake of brevity.

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Gross necropsy examination of the rats of the top dose level indicated the grossly visible target organs to include the liver, vascular system, respiratory system, and lymphoid organs; the general body condition was also consistently affected.

Microscopic examination of tissues from rats dying during the study or killed after 2 years revealed the following treatment-related affects:

Liver. The liver was the organ most consistently affected, and rats given 0.1 or 0.01 μg/kg/day had multiple hepatocellular degenerative, inflammatory, and necrotic changes noted upon light microscopy. These hepatic changes, which were more extensive in females than in males, were characterized by cytomegaly, distortion of

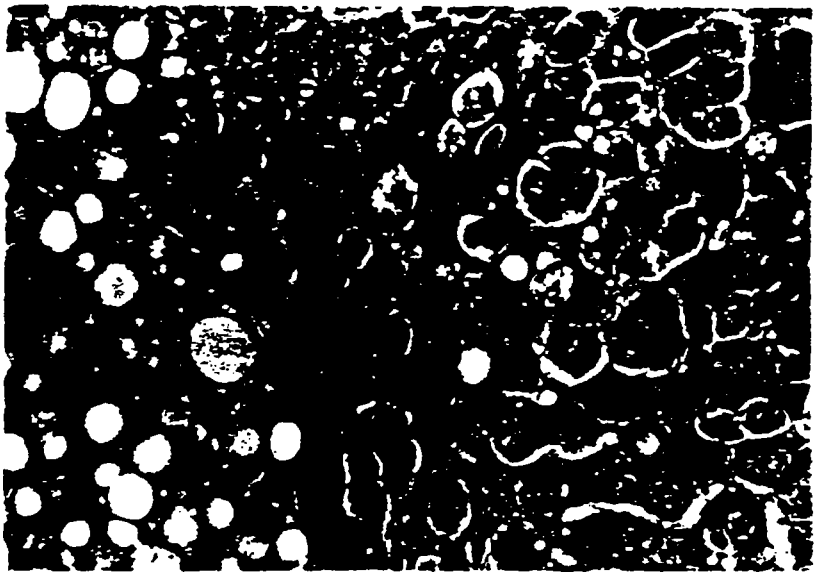


FIG. 1. Lesion classified morphologically as hepatocellular carcinoma in liver of rat given 0.1 μg of TCDD/kg/day. Note adjacent fibrosis, inflammation, and fatty infiltration on left. H & E stain. ×200.

lobular pattern, and resultant atrophy of hepatic cords, cytoplasmic vacuolization, fatty metamorphosis, altered tinctorial properties with increased basophilia, hepatic necrosis and inflammation, multinucleated hepatocytes, and foci or areas of hepatocellular alterations. They were accompanied by increased aggregates of pigment, bile duct hyperplasia, and some increase in fibrosis and periportal inflammation. During the latter phase of the study and at the terminal necropsy the females given 0.1 μg/kg/day also had hepatocellular proliferative lesions classified morphologically as hepatocellular carcinomas (Fig. 1) and hyperplastic (neoplastic) nodules. There was no evidence of metastasis of any liver neoplasms. Female rats given 0.01 μg/kg/day also had an increased incidence of these hepatocellular hyperplastic nodules. Upon examination using light microscopy, livers of female rats given 0.001 μg/kg/day had a statistical increase above the background incidence of foci or larger area of slight hepatocellular alteration (swollen hepatocytes). However, in male rats given 0.001 μg/kg/day, there was a statistically significant decrease in the number of livers with an area of hepatocellular alteration of this type.

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As part of the ultrastructural evaluation of hepatocytes, light microscopy of toluidine blue-stained sections of liver from females given 0.1 μg/kg/day revealed an increased number of individual hepatocytes containing large accumulations of lipid droplets. Ultrastructural evaluation by electron microscopy of liver sections from this high dose



FIG. 2. Hepatocyte from female rat given 0.1 μg of TCDD/kg/day for 2 years. Note disorientation of RER and focal cytoplasmic vacuolization. Uranyl acetate–lead citrate stain. ×3350.

level revealed the most consistent change to be in the rough endoplasmic reticulum (RER), which appeared to be undergoing proliferation with some distortion and fragmentation (Fig. 2). Smooth endoplasmic reticulum (SER) and mitochondrial structures were within the range of variation observed in the control sections. Other changes noted at this high dose level included focal areas of cytoplasmic vacuolization, increased lysosomal activity with residual body formation, and an occasional multinucleated hepatocyte (Fig. 3). Upon ultrastructural examination of hepatocytes from

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rats of the 0.01-μg/kg/day dose level, the most notable change was limited to a lesser degree of proliferation and disorientation of the RER and some proliferation of SER (Fig. 4). There was some slight increase in the number of individual hepatocytes with lipid droplet accumulations.

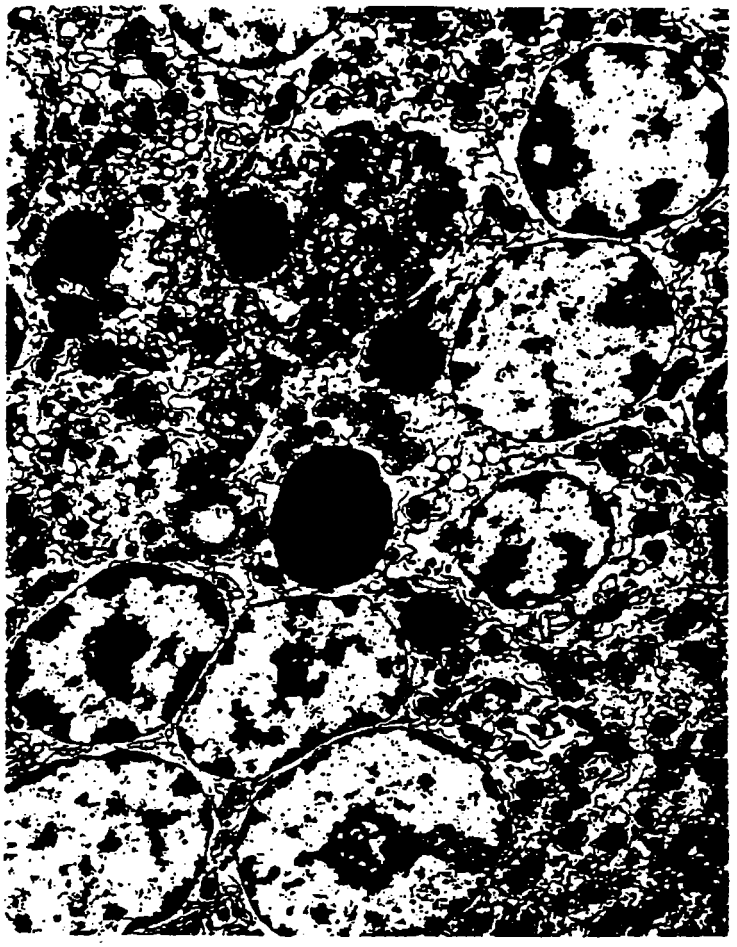


FIG. 3. Multinucleated hepatocyte from female rat given 0.1 μg of TCDD/kg/day for 2 years. Note focal area of cytoplasmic degeneration and distribution of RER. Uranyl acetate–lead citrate stain. ×1620.

The hepatocytes of female rats given 0.001 μg/kg/day were ultrastructurally within the limits of variation seen in the controls (Fig. 5). There was no general increase in the lipid droplet content, but an occasional cell contained increased numbers of lipid droplets.

Lymphoreticular tissues. Treatment-related effects, noted only in females of the 0.1-μg/kg/day dose level, included isolated occurrences of thymic atrophy and/or splenic atrophy.

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Respiratory system. Treatment-related effects were noted in both males and females at the 0.1-μg/kg/day dose level but were much more extensive in the female rats and included an increased incidence of focal alveolar hyperplasia (Fig. 6), aggregates of hematogenous pigment in lung and thoracic lymph nodes, focal

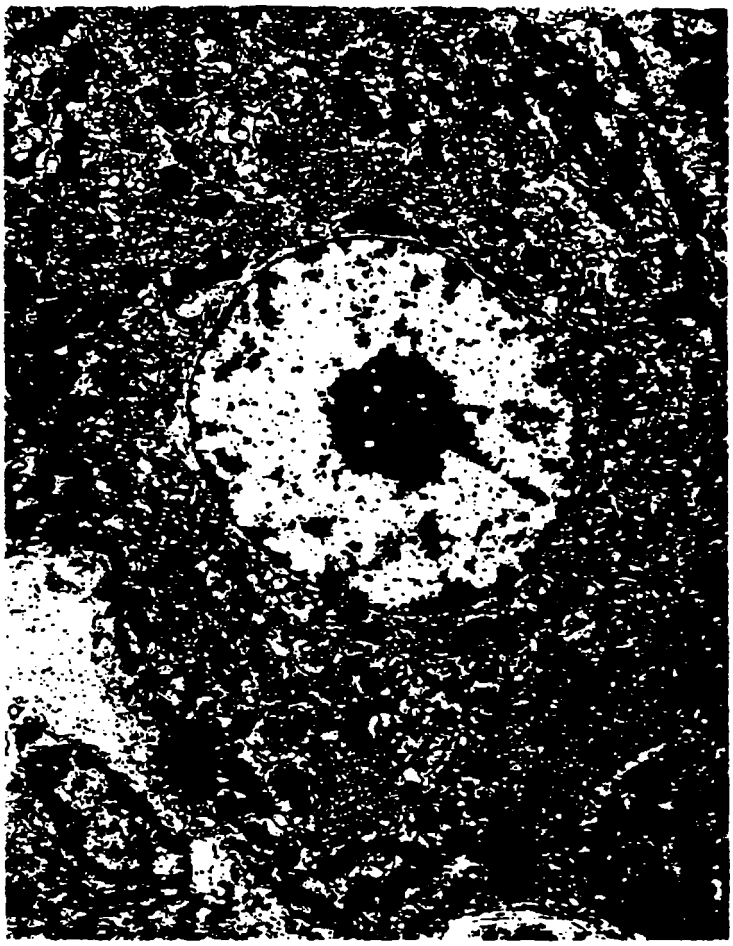


FIG. 4. Hepatocyte from female rat given 0.01 μg of TCDD/kg/day for 2 years. Note proliferation of SER and disorientation of RER. Uranyl acetate–lead citrate stain. ×2070.

accumulation of alveolar macrophages and cholesterol clefts, pulmonary edema, focal interstitial inflammation and fibrosis, keratinizing squamous metaplasia, or squamous cell carcinoma formation (Fig. 7) within the lung. Focal alveolar hyperplasia was also increased in females given 0.01 μg/kg/day. The lower dose level of 0.001 μg/kg/day had no discernible effect on the tissues of the respiratory system.

Cardiovascular system. Effects probably related to the ingestion of 0.1 μg/kg/day included an apparent increase in the incidence of hemorrhage in the brain and possibly

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spinal cord of females, an increase above the background incidence rate of mesenteric/thoracic periarteritis with accompanying changes, such as thrombosis and hematoma formations in both males and females, and an increase above the background incidence of myocardial degenerative changes (females only). At the 0.01-



FIG. 5. Hepatocyte from female rat given 0.001 μg of TCDD/kg/day for 2 years. Morphology within normal limits of variation seen in controls. Uranyl acetate–lead citrate stain. ×4100.

μg/kg/day dose level, probable treatment-related lesions were limited to an increase above background incidence of periarteritis and thrombosis of testicular or thoracic/mediastinal vessels of male rats. There were no alterations considered related to treatment with 0.001 μg/kg/day.

Reproductive system and mammary gland. Female rats given 0.1 μg/kg/day had a statistically decreased incidence of uterine changes, including endometrial hyperplasia,

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cyst formation, and adenomatous polyp formation. This same group of high dose level female rats also had a significantly decreased incidence of subcutaneous mammary tumors. These observations correlated well with a decreased incidence of pituitary

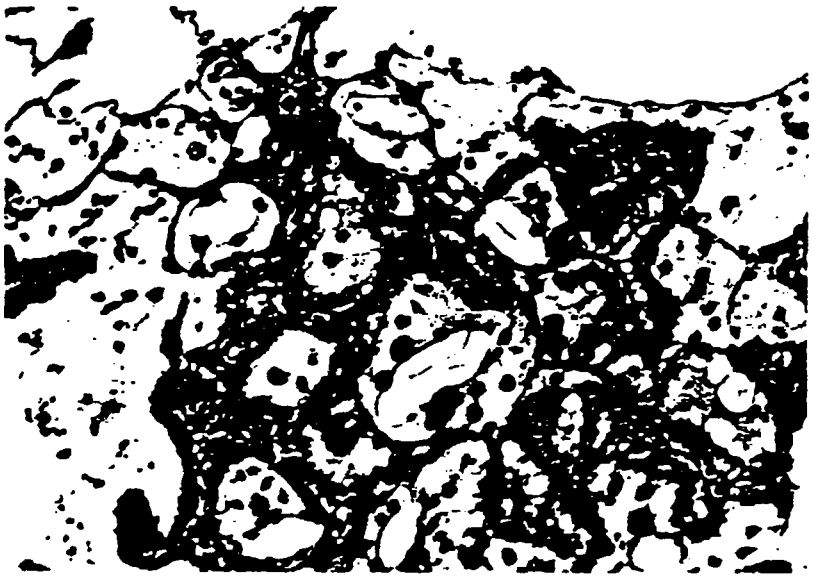


FIG. 6. Focal alveolar hyperplasia near terminal bronchiole within lung of rat given 0.1 μg of TCDD/kg/day. H & E stain. ×100.



FIG. 7. Lesion within lung of rat given 0.1 μg of TCDD/kg/day classified morphologically as squamous cell carcinoma. Note accumulation of keratinized material within lesion. H & E stain. ×100.

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adenomas noted in this same high dose level of female rats. There were no discernible effects in female rats given 0.01 or 0.001 μg/kg/day.

The reproductive organs of male rats appeared to be unaffected by these dose levels, with similar degenerative, inflammatory, and proliferative lesions in all treated and control groups.

Endocrine organs. Female but not male rats given 0.1 μg/kg/day had a significantly decreased incidence of pituitary changes, including hemangiectasis and adenoma formation. Adrenal changes noted at this high dose level included a decreased incidence of medullary hyperplastic nodule formation (males and females), a decreased incidence of pheochromocytoma formation (males), an increased incidence of cortical necrosis and hemorrhage (females), and an increased incidence of adrenal hematocyst formation (males). A statistical increase in the incidence of adrenal cortical adenomas noted for males given 0.1 μg/kg/day may have been the result of normal biological variation of the incidence of this tumor, which does occur spontaneously in this strain of rat (approximate 10% incidence in the control group of female rats used in this study).

The pancreas of male rats given 0.1 μg/kg/day had a statistical decrease in the incidence of acinar adenoma formation. A statistically increased incidence of fibrosis of atrophic pancreatic tissue noted in the group of females given 0.1 μg/kg/day may or may not have been associated with the increased incidence of periarteritis noted in this group. The thyroid glands of the high dose group of male rats appeared to have a low incidence of various follicular changes that may or may not have been related to treatment; this included isolated cases of follicular cyst or microcyst formation, follicular adenoma, or follicular adenocarcinoma formation. The parathyroid gland was unaffected by treatment, except for a *decrease* in secondary parathyroid hyperplasia as a result of the decrease in severity of chronic renal disease of the males given 0.1 μg/kg/day.

Gastrointestinal system. A wide variety of degenerative or inflammatory lesions occurred in all control and treated groups, with no indications of a direct treatment-related effect in the salivary glands, esophagus, stomach, small intestine, or large intestine. However, the group of male rats given 0.1 μg/kg/day had a statistical increase above background incidence of stratified squamous cell carcinomas of the tongue, which were considered to be probably related to treatment. There was also a statistically significant increase in the incidence of squamous cell carcinomas of the hard palate/nasal turbinate region of male and female rats given 0.1 μg/kg/day. Historically, squamous cell carcinomas of the tongue and hard palate/turbinates have occurred at a spontaneous incidence rate of 1 to 3% in this strain of rat. It appears as if treatment with 0.1 μg/kg/day increased the incidence of this type of neoplasm. A secondary effect of treatment was noted in the stomach only of the high dose group of males, in which there was a decrease in the incidence rate of mineralization of the gastric muscularis and mucosa. This was secondary to the decreased incidence and severity of chronic renal disease and uremia in this high dose group of male rats.

Nervous system. The only observation considered as probably related to treatment was the increased incidence of focal hemorrhage in the brain (and possibly spinal cord) of female rats given 0.1 μg/kg/day. This was described previously in the description of the cardiovascular system. All groups of rats had the expected spectrum of degenerative, inflammatory, and proliferative lesions considered spontaneous in origin.

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Urinary system. There appeared to be a *decrease* in the severity of the chronic nephropathy affecting the kidneys of the male rats given 0.1 μg/kg/day. Other degenerative, inflammatory, and proliferative changes occurred in the kidneys or urogenital tract of control or treated groups, with no observations considered related to treatment.

Musculoskeletal system, eye, and miscellaneous tissues. Various degenerative, inflammatory, or proliferative lesions occurred in a scattered pattern in all treated and control groups, with no indication of a treatment-related effect. No toxicologic significance was attached to the statistical decrease in the incidence rate of subcutaneous benign tumors noted in males given 0.001 μg/kg/day.

Organ Weights

Statistically significant differences in terminal organ weights considered related to treatment included (1) an increase in liver weight calculated on an absolute basis (males given 0.1 or 0.01 μg/kg/day, females given 0.1 μg/kg/day) and on a relative basis of liver/body ratio (females given 0.1 or 0.01 μg/kg/day) and (2) a decrease in the absolute weight of the thymus of females given 0.1 μg/kg/day. Additional changes in organ weights were considered to be secondary to decreased body weights due to treatment with 0.1 μg/kg/day.

TCDD Content of Tissues

Results of analysis of samples of fat and liver collected at terminal necropsy of female rats after 2 years of treatment indicated that rats given 0.1 μg/kg/day had an average TCDD content of 8100 ppt in the fat and 24,000 ppt in the liver. Rats given 0.01 μg/kg/day had an average TCDD content of 1700 ppt in the fat and 5100 ppt in the liver. Rats given 0.001 μg/kg/day had an average of 540 ppt of TCDD in the fat and also in the liver.

DISCUSSION

The findings of this chronic toxicity study on TCDD in rats are an extension of the studies of shorter duration reported previously from this laboratory (Kociba *et al.*, 1976). Continuous ingestion of diets containing approximately 2200 ppt of TCDD (0.1 μg of TCDD/kg/day) for 2 years caused multiple toxicologic effects, including increased mortality, decreased body weight gain, slight depression of certain hematologic parameters, increased urinary excretion of porphyrins and δ-ALA, increased serum activities of AP, GGT, and SGPT, and morphological changes primarily of the hepatic, lymphoid, respiratory, and vascular tissues of the body. This high dose level of 0.1 μg/kg/day also caused an increase in the incidence of hepatocellular carcinomas of the liver (females only) and squamous cell carcinomas of the lung, hard palate/nasal turbinates, or tongue. The occurrence of numerous age-related lesions usually encountered in this strain of rat, including tumors of the pituitary, uterus, mammary gland, pancreas, and adrenal gland was reduced at the high dose level. Also reduced was the incidence and severity of chronic renal disease in the aged male rats. Female rats given this high dose level for 2 years had 24,000 ppt present in the liver. This compares with 34,600 ppt of TCDD present in the liver of female rats given this

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same dose level for 13 weeks (Kociba *et al.*, 1976) and indicates steady state concentrations were achieved during the early phase of this 2-year study.

Ingestion of the intermediate dose level of 0.01 μg/kg/day (~210 ppt in the diet) caused a lesser degree of toxicity. The primary effects noted at this dose level included (1) increased urinary excretion of porphyrins (females), (2) liver toxicity, including an increased incidence of hepatocellular nodules, and (3) increased incidence of focal alveolar hyperplasia in the lungs. Terminal liver and fat content of TCDD averaged 5100, and 1700 ppt, respectively. This compares with 3700 ppt present in the liver after 13 weeks of treatment with this dose level (Kociba *et al.*, 1976).

Lifetime ingestion of 0.001 μg/kg/day (~22 ppt in the diet) caused no effects considered to be of any toxicological significance. Light microscopy of livers from females of this group indicated a statistical increase above the background incidence of swollen hepatocytes; conversely, the livers of the males of the group had a decreased incidence of this observation. When liver tissue was examined using electron microscopy, the hepatocytes from the females were within the limits of variation seen in the controls with an occasional hepatocyte containing increased lipid droplets. The liver and fat each contained 540 ppt of TCDD at the end of the lifetime ingestion of 0.001 μg/kg/day.

If the results of this lifetime study in rats are compared to the preliminary results of the study in rats by Van Miller *et al.* (1977), it will be noted that both studies report neoplastic responses in the lung and liver of rats maintained for extended periods of time on high doses of TCDD. In the preliminary report by Van Miller *et al.* (1977), 5000 ppt produced both liver and lung neoplasms, while in this study, 2200 ppt produced both liver and lung neoplasms.

Thus, there is agreement between the results obtained in both studies at higher dose levels of 2200 to 5000 ppt of TCDD in the diet. However, at lower dose levels, there are differences in the two studies, with Van Miller *et al.* (1977) reporting a diverse spectrum of neoplasms in rats given as low as 5 ppt of TCDD, based on a zero incidence of neoplasms in a total of 50 control rats examined in their study. Conversely, in this study, there was no carcinogenic response in rats given 210 or 22 ppt of TCDD for 2 years. DiGiovanni *et al.* (1977) reported TCDD to be only a weak tumor initiator in studies of mouse skin carcinogenesis with DMBA.

In summary, data collected in the study reported herein indicate that doses sufficient to induce severe toxicity increased the incidence of some types of neoplasms in rats, while reducing the incidence of other types. No increase in neoplasms occurred in rats receiving sufficient TCDD during the 2-year study to induce slight or no manifestations of toxicity.

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Part I

During the years from 1965 through 1969, a chemical plant producing herbicides - pentachlorophenol and 2,4,5-trichlorophenoxyacetic acid derivatives - reported a mass outbreak of acne chlorina. The number of persons stricken, 78, is one of the largest ever recorded. Furthermore, some of our patients were also stricken by a complete intoxication manifested by liver lesions, by a deficiency in porphyrin, fat and protein metabolism and by nervous and physical changes. In two cases, the intoxication was fatal. This part of the research will deal primarily with skin symptoms and the internal and neurological changes will be discussed in a subsequent work [23] and in more detail in another work [32].

The acne forming dermatoses are caused in most cases by crude oil derivatives - known as acne oleosa - and less often by tar and its derivatives - in these cases it is acne picea. In contrast to those acnes, acne chlorina is relatively very rare because it is only caused by a small number of certain chlorinated hydrocarbons which are produced and used only on a limited scale. Acne chlorina also differs from other acne forming dermatoses by its localization, clinical picture and course.

Mass occurrences of acne chlorina have been recorded during several time periods which are separated by decades, in accord with industrial and chemical development and with the gradual introduction into production of new chlorinated hydrocarbons.

The first cases were described by Herxheimer [18] in 1899 in the electrolytic production of sodium chloride and sodium hydroxid from brine. Herxheimer assumed that the illness was caused by the inhalation of chlorine and by its excretion through membranes and he therefore named the disease acne chlorina, i.e., chloracne. The French authors Thibierge [43,44] and Hallopeau [16] were of the same opinion as to the incidence of this disease. Furthermore, Hallopeau thought that the black coloration of the comedones

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was caused by some lead compound that formed on the chamber's lead walls as a result of a reaction with chlorine. Renon [35] also connected chloracne with lung tuberculosis in one of his patients and ascribed both diseases to gaseous chlorine.

However, Hirsch [20] concluded in that same year that the cause must be a solid substance which formed on the anodes during electrolysis because exposure to chlorine gas alone did not cause the disease. This finding was supported by Bettmann [2] who observed 21 patients who were engaged in the production of hydrochloric acid and who were in contact with chlorinated tar compounds but not with chlorine gas.

Lehmann [26], in 1903, concluded that the actual causes of the disease were chlorinated products such as hexachlorobenzene, pentachlorobenzoic acid and hexachloroethane which formed on the carbon anode during electrolysis. Because similar cases of acne chlorina were also observed in the production and processing of *p*-nitrochlorobenzene, W. Lehmann [27] concluded that acne chlorina was caused primarily by chlorobenzenes. As soon as carbon anodes were replaced by magnetite anodes, the incidence of acne during electrolysis ceased.

Before World War I, Wahle [46] observed acne chlorina in two chemists which was probably caused by a chlorine compound - phenyler dioxide - not identified or known in more detail at that time.

The next wave of the disease's incidence occurred at the end of World War I during the production of chlorinated naphthalenes or perchloronaphthalenes. In this period the disease was given an additional new name - Pernakrankheit (Wauer [42]). Chlorinated naphthalenes and diphenyls with a waxy consistency, also known as halowaxes, have some very valuable properties such as waterproofness, flame resistance and acid resistance. These properties earmarked these materials for the production of protective gas masks and other military equipment.

A new increase in the incidence of the disease occurred in the nineteen-twenties during the use of chlorinated naphthalenes

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as an insulating material in the mining industry (Teleky [42]). Acne chlorina also appeared in the electrolytic production of chlorine when insulating coatings of tar were used. This fact brought about numerous disagreements and questions as to whether the disease in those production processes was not actually acne picea. On the other hand, Teleky supported the contention that the tar acne could be caused by chlorinated hydrocarbons contaminating the tar.

The excellent dielectric properties of the chlorinated naphthalenes ("haftax", "halowax", "nibrene") led to their wide utilization in the production of cables and condensers and another mass occurrence of the disease was produced in the nineteen-thirtie when the U.S.A. and other countries recorded hundreds of stricken individuals. Holtzmann [22] also noted the occurrence of acne among the family members of workers employed in the production of condensers. These family members handled and washed work clothes contaminated with the chlorinated naphthalenes. Based on these observations, Holtzmann corrected his previous original conclusions and stated that the chloracne was actually tar acne and judged the effect of halowaxes to be that of an external acnegenic cause.

Ormsby [31] observed acne chlorina with the use of a fungicide used in the impregnation of lumber (sodium tetrachloro-*o*-phenylphenolate). Jones and Alden [24], Schwartz [39] and later many others also observed total disorders in their patients and Flinn and Jarvik [8] noted a death due to yellow liver atrophy. In 1938, Mayers and his coworkers [28] were unsuccessful in clarifying the mechanism of occurrence of dermatitides and acne chlorina in their patients with the aid of epicutaneous tests - the tests were negative.

During World War II, chlorinated naphthalenes were used for the impregnation of ships' hulls when it was discovered that these substances could protect ships from damage of magnetic mines. The use of halowaxes increased significantly, especially in the electical industry. American authors gave acne chlorina another name,

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"cable rash", because of the occupation (cable production) of the patients (Good and coworkers [14], Schwartz [40], Morris [29]). At that time, massive outbreaks of acne and several deaths were recorded. After World War II, the incidence of acne did not subside because the undesirable experiences with the chlorinated naphthalenes did not prevent their use in the electrical industry for some time. An interesting observation was recorded by Herzberg [19] in 1947. He observed seven cases of acne chlorina in patients who had ingested chlorinated paraffins in the form of a food shortening. This prompted Herzberg to conclude that acne chlorina must occur after the internal ingestion of chlorinated hydrocarbons. Similarly, Fuchs [9] also noted the affliction of three children and their mothers after they had eaten potatoes fried in the "paraffin". Winkler [48] recorded 10 cases of dermatitis and 13 cases of acne chlorina among 23 workers in a condenser factory that used nibrene in the production sequence. From these cases Winkler concluded that "chlorinated hydrocarbons cause the formation of reagents that in turn cause an increased secretion from lubricating glands". In 1949 Gavrilova [10] warned of the photosensitization effect of polychloronaphthalenes. In recent years, plastics have replaced chlorinated naphthalenes in industrial use.

Even today the production of sodium hydroxide may be a source of acne chlorina. We have observed more cases between 1950 and 1965. The main causes of acne chlorina in these cases have been the combination of chlorine gas with the hydrocarbons in the tar gaskets and fittings on the electrolytic containers and tubs and with the mineral oils which were used to protect the electrode contacts. The authors noted several cases of acne chlorina among laboratory workers after the use of nibrene in the electrical industry in the 1950's.

Furthermore, the acnegenic effects of the mineral oil based coolant mixtures used in metal working increase when chlorinated hydrocarbons are added. Therefore, some cases of acne oleosa were observed which exhibited a partial acne chlorina character (more

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distinct follicular hyperkeratosis, less inflammation, cyst formation and atypical localization).

The last wave of the disease's occurrence was recorded in the 1950's. This outbreak was noted among persons engaged in the production of chlorinated phenol type insecticides and herbicides. From 1950 through 1951, Baader and Bauer [1] and Brinkmann [7] published information on the occurrence of acne chlorina which involved the complete internal, nervous and psychic symptoms among 17 workers engaged in the production of pentachlorophenol. Several years later, Hergt [17], Oettel [30] and Hoffmann [21] observed a total of 80 afflicted workers who were employed in the production of 2,4,5-trichlorophenol by the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene. From 1954 through 1956, Kimmig and Schultz [25] recorded 31 cases of acne chlorina in one Hamburg (Germany) plant that produced 2,4,5-trichlorophenoxyacetic acid and its esters. By very detailed research and tests, these authors have shown that acne chlorina and total intoxication with damage to the internal organs, mainly to the liver, with psychovegetative disorders were not caused by the basic chlorinated hydrocarbons, i.e., tetrachlorobenzene, trichlorophenol and trichlorophenoxyacetic acid, but rather were caused by a ballast substance concentrated in these compounds in small quantities, namely, 2,3,6,7-tetrachlorodibenzodioxine. These authors contributed significantly with their other experimental works to the question of what substances cause acne chlorina and how these substances act.

In 1964, Bleiberg and coworkers [3] observed 29 cases of acne chlorina among persons engaged in the production of 2,4,5-trichlorophenol and 2,4-dichlorophenol. In eleven of these cases they also diagnosed symptoms of porphyria cutanea tarda. In 1957 Bowen and coworkers [4] observed acne chlorina among workers employed in the production of DDT from trichlorobenzene and hexachlorobenzene. The fates of 53 patients who in 1953 had worked in the alkaline hydrolysis of tetrachlorobenzene to trichlorophenol were described only last year by Goldmann [11]. In addition to acne, Goldmann also diagnosed in his patients bronchitides and

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disorders of the liver, kidney, myocardium and nervous system. The author described the death of a patient from necrosis of the pancreas as a special case. Furthermore, he also observed dermatitis in a nurse who had had contact only with experimental animals. Finally, he diagnosed acne chlorina in the fourteen year old son of an employee whose disease was contracted only from the household environment - by wearing his father's contaminated scarf and by using his father's towel.

Actual observations

The process called PCP (pentachlorophenol) which caused mass damage in Czechoslovakia was, as far as production is concerned, identical to the process from which Kimmig and Schulz drew their patients for observation. The final products of this process were sodium pentachlorophenolate, 2,4,5-tricholorphenoxyacetic acid and the latter's sodium salt and butyl ester. The primary raw material was technical grade trichlorobenzene which was produced in a neighboring building for the production of hexachlorocyclohexane (HCH) and lindane. The table shows the entire production scheme (Table I). The chlorination of trichlorobenzene produced tetrachlorobenzene and hexachlorobenzene. Sodium pentachlorophenolate was formed by the alkaline hydrolysis of hexachlorobenzene. Sodium trichlorophenolate was produced by the alkaline hydrolysis of tetrachlorobenzene. The hydrolysis of tetrachlorobenzene with sodium hydroxide in the presence of methanol took place in an autoclave at 190°C and at 45 atm of pressure for one hour. After cooling, the methanol was distilled from the hydrolysate, the hydrolysate was diluted with water to a 25% concentration and it was then syphoned into a storage tank. The condensation of sodium trichlorophenolate with monochloroacetic acid produced sodium trichlorophenoxyacetate. Following cooling, centrifugation and flushing with water, the dried sodium trichlorophenoxyacetate was picked out by hand and placed in small barrels. Some of this product was used to produce the butyl ester of

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trichlorophenoxyacetic acid. This production took place in another building.

The production of both the primary products took place in a four story building with grated floors (WEMA grates) which was one reason why the entire building was contaminated with the high risk substances. The building was not sufficiently airtight, the local exhausts were in most cases improperly installed and hermetization and mechanization were insufficient. Initially, the sodium tetrachlorophenolate [sic] was escaping from the centrifuge into the work space in the form of a mist. A series of tasks such as the removal of solid substances and the pumping of liquids were performed by hand. The workers were dressed in linen work clothes. The thorough cleaning and frequent changing of the work clothing was impossible to guarantee over the entire interval of production. Rubber gloves and respirators were used at some work sites. The conditions surrounding the production of the butyl ester of trichlorophenoxyacetic acid were the same as those described above.

Kimmig and Schulz [25] found that the main source of acnegenic hydrocarbons was that segment of the production process in which sodium trichlorophenolate is formed by the alkaline hydrolysis of tetrachlorobenzene in the presence of methanol. A coworker of these authors, Doctor (of Chemistry) Sorge, had deduced on theoretical grounds that if this reaction were conducted at 190°C and 45 atm of pressure, chlorinated hydrocarbon by-products such as polychlorodibenzo-*p*-dioxines, polychlorodibenzofurans, polychlorodiphenyl ethers and polychlorodiphenyl oxides may be formed in small amounts (Schulz [36]). Sorge also prepared some of these substances by his synthetic process.

Animal tests carried out by the authors (Schulz and Kimmig) with tetrachlorodibenzofuran and 2,3,6,7-tetrachlorodibenzodioxine have proved an extraordinarily high toxicity for these substances. A simple smear of a 0.1% solution of tri- and tetrachlorodibenzofuran on a small area of a rabbit's auricle caused heavy liver

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damage and death of the test animal within 2 to 3 weeks (Schulz [36]). 2,3,6,7-Tetrachlorodibenzodioxine, which also has very strong acnegenic effects, was proved to be far more toxic. Only then was Dr. Sorge successful in the identification of this enormously toxic substance--in the production process and in the final product. He proved that this toxic substance is formed only at certain temperatures and pressures. If during the alkaline hydrolysis of tetrachlorobenzene to sodium trichlorophenolate the temperature is reduced below 153°C, the toxic substances do not form. However, at this low temperature, the reaction time is extended from one to five hours and the productivity is obviously lower. Furthermore, it was proved that the condensation of two molecules of trichlorophenolate to give tetrachlorodibenzodioxine is an exothermic reaction. Several explosions of autoclaves with injuries and the lethal intoxication of workers were reported to be the result of this reaction in the German Democratic Republic (East Germany) (Schulz [38], Goldmann [11,12]). Tetrachlorodibenzodioxine was also identified in the Czechoslovakian production process and in the products as well.

The first two cases of acne chlorina were noted in 1965 in two technicians who operated the production machinery. It was assumed at that time that these two cases were caused by careless work and an undeveloped production technology under semiproduction conditions. After one year without a reported case and during which production was temporarily halted, a mass outbreak of 78 cases was recorded within the following three years. Finally, after a detailed investigation and study of the entire problem, the process was terminated and production ceased in 1968. Workers from all stages of production were stricken. At that time, it was impossible to determine which production stage was the most risky because most of the workers were involved in several stages at various locations in the plant within a relatively short time. Workers directly involved in production as well as maintenance personnel and workers digging sewage ditches were stricken. Later a research scientist working to isolate the acnegenic ballast

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substances from the products of the individual production phases was even stricken. His research work was carried out outside the production plant in a research institute's laboratory. In addition to the 78 patients with dangerous symptoms of acne chlorina or porphyria, other workers in this plant had symptoms of acne which were, however, symptoms of juvenile acne or of a similar character and could not therefore be counted as an occupational disease.

With the exception of two female laboratory technicians, the patients consisted of males from 18 to 57 years of age (4 persons below 20, 46 persons from 21 to 30, 11 persons from 31 to 40, 10 persons from 41-50 and 7 persons from 51 to 57). Therefore, the acne chlorina patients were mostly young people. On the other hand, more than half the patients with porphyria cutanea tarda were older than 40 years.

Generally, the disease followed a normal course as described by other authors. In only six patients did the disease begin suddenly as acute solar dermatitis, i.e., by erythrema and edema in regions exposed to solar radiation and continued by acne symptoms (Photographs 1 and 2), as observed by Goldmann [12], Grimmer [15], Braun [6] and before these authors, also by Touraine and coworkers [45]. Therefore, chlorinated phenols in some cases have similar photodynamic effects as the chloronaphthalenes described by Gavrilova [10]. Erythema and edema in these patients were obvious mainly on their auricles. Sometimes, small blisters in thick groupings were observed there.

In all the other patients the acne developed slowly. Among the less severe cases the disease was limited to single or densely grouped comedones, conditoned by follicular hyperkeratosis, which were so small and sometimes so numerous that they appeared to be a slate grayish-brown color, like dirty spots on the skin. These symptoms were noted mainly on the face, with maximum occurrence above the cheek bones (Photograph 3). In the more severe cases, the comedones gradually developed into small white cysts similar

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to milia, although with black centers, and into large cysts ranging from the size of a pea to that of a nut (Photographs 4, 5 and 6). As opposed to other acne forming dermatoses, the described symptoms and manifestations mainly lacked signs of an infection. Only in the more severe cases and rather in the vicinity of large cysts were some reactive inflammations, infections and abscesses noted. The emptied cysts and abscesses left behind large atrophied scars with raised edges and with bridges of intact skin under which a probe could be inserted (Photograph 7). This basic clinical picture was supplemented by diffused hyperpigmentation in the face and by papulopustules on the torso and extremities. Patients who were found to suffer from a disorder in porphyrin metabolism had symptoms of porphyria cutanea tarda in regions exposed to the sun (hyperpigmentation and hypertrichosis, blisters, excoriation and small scars). However, in this group there were two patients with prophyria but almost without any acne at all.

Histological investigation is not much help in diagnosis. The symptoms begin with hyperkeratosis at the mouth of the follicle After one to three weeks, the follicle changes into a cystic formation filled with a horny substance [keratin?] (Photograph 11) and to a small extent with sebum. However, the epidermal changes are small. After the rupture of the full follicle, the contents of the cyst penetrate into the surrounding tissue and a granuloma of foreign bodies forms around the area (Photograph 12).

The disease normally starts on the face above the cheek bones. However, in 17 patients the disease started in an unconventional manner by papulopustular eruptions on the extremities, primarily on the lower extremities. This characteristic, resembling more acne oleosa, remained in some of the patients for the entire duration of their illness.

The most common localizations of acne chlorina were the cheeks temples and to a lesser extent, the entire face. Eyebrows, eyelids, the perioral region and the scalp and hair area remained free

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Other predilected areas were the auricles, the back of the neck, the back and the chest in a seborrheic localization and the genitals. The auricles and genitals and especially the scrotum were stricken by the formation of large cysts (Photographs 8 and 9) Among the five most seriously ill patients, the disease spread all over the body and had a discouraging effect that was heightened by the disease's usual persistence, the long term healing and by medical helplessness (Photographs 10 and 7). The contents of the cysts produced a repulsive and rancid odor. For some of the patients the disease disrupted their social and family relations and caused severe depressions.

Some authors, such as Braun [6], emphasize itching as the initial symptom, but we noted this symptom in only three cases.

In 20 patients with extensive symptoms, the occurrence of pustules, cysts and abscesses was also noted in axillae although this location was not mentioned by other authors. Because of the great number of follicles in the axillae and the similarity of the axillary skin to that of the genitals and for several other reasons, this finding appears to be just as valid as localization on the genitals and the genital area. On the other hand, it is surprising that acne chlorina does not strike at the hairline or scalp. In the case of one patient, the acne symptoms disappeared in the facial areas when he grew a full beard while in other areas, the symptoms continued without a reduction in intensity. So far there is no explanation for this phenomenon.

Hypertrichosis or hyperpigmentation or both were determined in the faces of 19 patients without a laboratory diagnosis of a deficiency in porphyrin metabolism. Bleiberg [3] made similar observations. These symptoms receded simultaneously with the healing of acne chlorina.

~~More younger persons suffered from acne chlorina than older persons.~~* This study group contained 50 persons (85%) below 30

~~*Translator's note: Beginning of this sentence illegible.~~

*Many authors believe that younger persons have a greater tendency to develop acne chlorina than older individuals. (Correction by NIH Translation Unit

years of age. However, this fact cannot be used as a hard and fast rule or to support the findings because the production plant under study employed mostly young workers. The preillness medical history among the patients did not indicate any significant predisposing factors and no incidence of serious illnesses, intoxication or ~~etiolation~~*. Furthermore, no relation to hair color, eye or complexion color, to the degree of body hair, to the perspiration of skin oils or to hereditary follicular hyperkeratosis and other skin diseases was noted. Only one patient reported a more severe prior case of acne juvenilis, 12 patients reported mild cases during puberty and 14 patients reported only insignificant acne juvenilis symptoms. 18 patients also had some infrequent contact with mineral oils either during the appearance of the symptoms or at some time in the past. These patients were mostly involved in maintenance work as mechanics. However none of them had ever had an oil acne in the past. Their illnesses following contact with tetrachlorodibenzodioxine were of the same character as the illnesses of the other patients. Their diseases were not similar to acne oleosa either in appearance or in localization.

The latent period from the initial contact with the risk substance to the appearnace of symptoms was quite varied. Some workers became ill after exposure of from several months to several years and the disease in their cases was rather mild although the indications pointed to a massive exposure. In other cases, severe damage occurred after a very short time of several weeks and after an apparently small exposure. For example, one of the most serious ill patients worked under the semiproduction conditions for only two and a half weeks before the outbreak of the disease (acne and porphyria cutanea tarda). After that he had no contact with the risk materials.

It is not clear whether individual predisposition or the level of personal and work hygiene is more responsible for the intensity

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~~*Editor's note: This may be a dubious choice. The Czech "etylismu transliterates to "etilism".~~

*alcoholism (Correction by NIH Translation Unit)

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of damage or latency of the disease. Most authors discount individual predispositon. However it is believed that the deciding factor must be the degree of actual exposure which is undoubtedly dependent on a series of other factors such as work discipline, personal discipline, adherence to preventive measures, frequency of changing work clothes and undergarments, the degree and regularity of hygienic decontamination, work site order and cleanliness, dust in the environment, etc.

These patients also had a series of subjective difficulties: tiredness, weakness in the lower extremities, muscle pains, sleepiness and insomnia, increased perspiration, lack of apetite, headaches and other disorders in the mental and sexual spheres. These complaints were more recurrent and more intense in patients with the more extensive skin symptoms. In more serious acne cases, a significant weight loss was also noted. It is our opinion that all these difficulties and symptoms are manifestations of a rather overall intoxication than of only the acne chlorina.

All the patients were admitted to a dispensary. Regular examinations of their dermatological, internal and neurological conditons were arranged. The patients who were still working were transferred to prevent further contact with any acnegenic factor. All contact with chlorinated hydrocarbons of any type was prevented even with those substances whose acengenic effects have not been thoroughly proved. Furthermore, all contact with mineral oils and tar and its products were prevented. The application of mineral based ointments was discounted for external therapy. The plant's medical doctors were cautioned against prescription of any internal medication with an acnegenic effect (iodine and bromium preparation corticoids and others). The patients were given detailed medical directions concerning their daily regimen, personal and work hygiene and secondary disease prevention and treatment.

All available medications for acne were utilized in the treatment. The expressions of comedones and the pressure relief of the contents of cysts and abscesses necessitated many periods of

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hospitalization and special ambulatory treatment hours attended by a specially trained nurse. No special results were observed as a result of repeated hospitalization. The best results were noted after the expression of comedones by the use of a sauna constructed for the patients by the factory on the author's recommendations. It was not possible to test the effects of vitamin A acid (retinoic acid) which has been praised by some authors in the medical literature. However, in agreement with the findings of other authors, it was determined that no local or internal medication exists which has an effective and permanent healing effect on acne chlorina. Some of the patients have been under observation for eight years and their disease still persists. Dr. Schulz's [38] patients have manifested continuous symptoms for more than ten years. Goldmann [11] has now been treating one patient with comedones and cysts on his penis and scrotum for 18 years! In most of the patients discussed by these authors, the symptoms and manifestations of the disease progressed and reached a maximum after about one year, even after transfer of the patient from the high risk work area. A regression takes place after two or three years at the earliest. In some isolated cases, the progress of a mild course of the disease was observed even after five years. However, the regression of the disease in all the patients was very slow and, it may be said, even lasts a number of years.

Of the 78 patients treated by the authors, 76 suffered from acne chlorina. Of these, 11 simultaneously had hepatic lesions with a deficiency in porphyrin metabolism. Two patients did not have acne chlorina. One of these two had porphyria and the second one died in 1966 from acute intoxication. Pentachlorophenol was considered to be the cause of death in that instance. When the toxicological analysis was carried out at that time, the investigators were unaware of tetrachlorodibenzodioxine and, therefore, it is our contention now that in that case, this highly toxic substance was primarily responsible for the acute intoxication.

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Gombos [13] mentioned one patient with acne chlorina and porphyria in connection with this production process.

The authors analyzed 55 patients internally and neurologically in detail. About half of these patients had a deficiency in lipid metabolism and in more than one-third, some minor biochemical deviations and mild hepatic lesions were noted in the first stages of the disease. In 17 patients, the authors found symptoms of central nervous system disorder, the majority with lesions of the peripheral neurons of the lower extremities (verified by EMG examination). In most of the patients, physical disorders, primarily an acute neurasthenic syndrome, were diagnosed. In addition to the death due to acute intoxication (pentachlorophenol? tetrachlorodibenzodioxine?), three more patients died. In one patient, the porphyria developed unusually rapidly into an arteriosclerosis of the brain with an atypical morphological diagnosis and demention. Two patients died from bronchogenic carcinoma All these patients with their complete symptoms are discussed in other papers [32,23].

Discussion

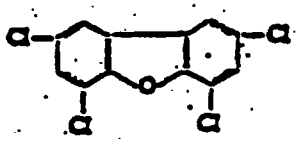
In agreement with Kimmig and Schulz, we cite the tetrachlorodibenzodioxine which is formed during the production process as the main reason for the skin symptoms as well as for all the other deficiencies mentioned and diagnosed in the authors' patients. This toxic compound was confirmed in considerable quantities in the final product (Arboricide E50), in the butyl trichlorophenoxyacetate and also in the mortar and wall paint of the factory building. Even adjacent rooms such as worker dressing and locker rooms were contaminated with this compound. Test rabbits placed into these rooms soon died.

In his monograph, Braun [5] cited the following chlorinated hydrocarbons as the then known causes of acne chlorina: chlorobenzenes with various degrees of chlorination, chlorinated phenols (sodium tetrachlorophenolate, pentachlorophenol), *para*-nitrochlorobenzene, sodium tetrachloro-*o*-phenylphenolate, chlorinated

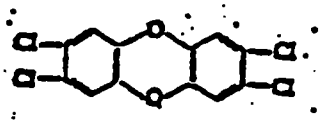
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naphthalenes, various chlorinated diphenyls, trichloroxydiphenyl, chlorinated diphenyl oxides, sodium *o*-(2-chlorophenyl)phenolate and others. However, Kimmit and Schulz [25] ascertained that pure pentachlorophenol and pure 2,4,5-trichlorophenoxyacetic acid do not have any acnegenic effects. Therefore, they checked the presumed effects of the other substances that were blamed for causing acne and found that neither pure nor chlorinated benzenes (from 1 to 8 chlorinated positions) nor chlorinated diphenyl-ethers have any acnegenic effects. A definite acnegenic effect was proved only for the following substances: chlorinated naphthalenes (5 and 6 chlorinated positions) in agreement with Shelley and Klingman [41], higher chlorinated dibenzofurans (diphenyl oxides) and 2,3,6,7-tetrachlorodibenzodioxine.

Therefore, it is possible and even probable that some chlorinated hydrocarbons that have been previously considered to have an acnegenic effect do not have this effect and the actual causes were other ballast chlorinated hydrocarbons contained in those hydrocarbons.



Tetrachlorodiphenylene oxide (tetrachlorodibenzofuran)



2,3,6,7-tetrachlorodibenzodioxine (tetrachlorodiphenyl dioxide)

In an animal experiment, Kimmig and Schulz found an exceptionally high toxicity for tetrachlorodibenzodioxine. In a local application on rabbits' auricles (keratosis test), this compound produced a strong reaction even at a concentration of 0.001%. Also, a skin application of this substance at the same concentration produced acne chlorina (Schulz [38]). Tetrachlorodibenzofuran was proved to be 10 to 20 times weaker. Chlorinated naphthalenes had a thousand times weaker effect. Generally, acne chlorina is caused by the direct contact of tetrachlorobenzodioxine with the skin. Absorption through the skin is not ruled out and an effect

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through the ingestive or respiration tracts is also conceded. However, in a pure ingestive experiment with a test animal, acne chlorina did not occur. However, the cases of acne chlorina contracted after the ingestion of chlorinated naphthalenes as food fat substitutes should not be forgotten [19,9].

Tetrachlorodibenzodioxine used internally has a strong hepatotoxic effect. A dose of 20 to 50 mg per kg of rabbit's body weight causes death in all the test animals by yellow liver atrophy.

Because of this enormous toxicity, all the preventive measures taken at the high risk processes in East Germany and Czechoslovakia were proved to be completely ineffective. The only dependable solution to that problem was the reduction of the reaction temperature and pressure during the alkaline hydrolysis of tetrachlorophenolate to sodium trichlorophenolate. In East Germany, the decontamination cost of the high risk areas and buildings was extremely high. One chemical plant attempted to use for other purposes a contaminated building which had not been decontaminated after the building had remained unused for two years, but new cases of acne chlorina were shortly reported. With normal decontamination procedures new outbreaks of the disease occurred and therefore, decontamination had to be carried out using special pressurized chemical protective suits with pressurized breathing apparatus because gas masks were not sufficient. Doors, windows, the wooden trim and other wooden building components had to be burned; the steel construction materials in the building had to be sandblasted; the plaster, facade and floors had to be torn out and cast into cement blocks together with the tubing, plumbing and other equipment. These blocks were then transported to the Atlantic Ocean and sunk. The disposition of those non-flammable materials by burying would have run the danger of contaminating the subterranean water supply. Only the most expensive components, autoclaves and some valves, were successfully decontaminated by repeated washing with special detergents.

The Czechoslovakian plants are still closed and their fate is still undecided. The patients are still under a continuous observation.

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[23] Symptoms of total intoxication in acne chlorina during the production of herbicides - in final preparation for printing.

[31] Discussion on paper by Jones [24]

[32] Health hazards to workers in production of sodium 2,4,5-trichlorophenoxyacetate.

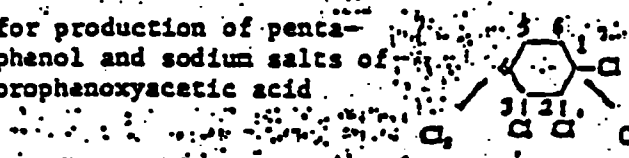
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TABLE I. Production Scheme.

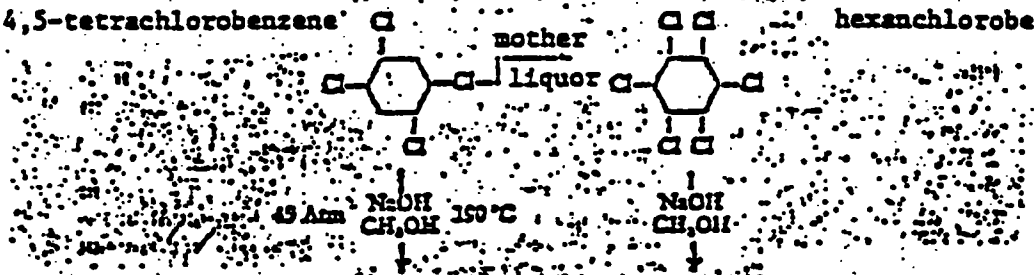
Plant for production of HCH (hexachlorocyclohexane)

1,2,3-trichlorobenzene and its isomers (1,3,5- and 1,2,40)

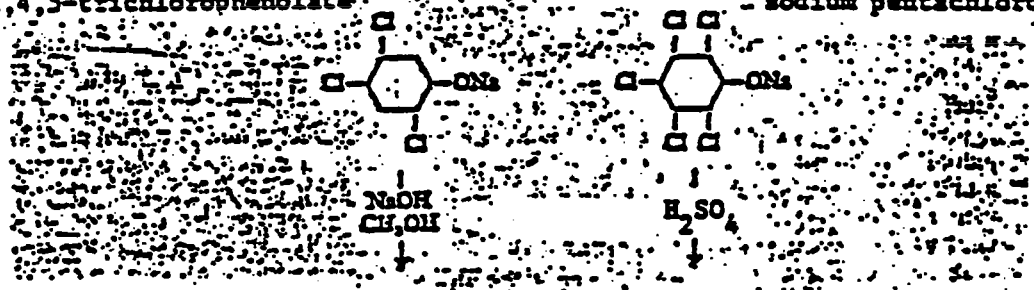
Plant for production of pentachlorophenol and sodium salts of trichlorophenoxyacetic acid



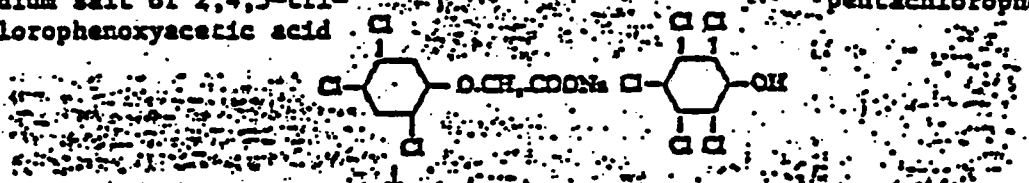
1,2,4,5-tetrachlorobenzene



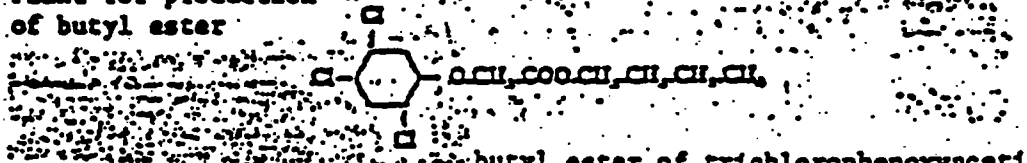
sodium 2,4,5-trichlorophenolate



Sodium salt of 2,4,5-trichlorophenoxyacetic acid



Plant for production of butyl ester



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и мутилирующими язвами по всему телу. Разнообразная клиническая картина документирована рядом фотографий. Двенадцать пострадавших имели поражения печени с признаками porphyria cutanea tarda, а у некоторых пациентов были обнаружены нервные и психические расстройства.

Оказалось, что кожные и общие повреждения были вызваны 2, 3, 6, 7 — тетрахлордибензодиоксином, который возникает при алкалическом гидролизе тетрахлорбензола в трихлорфенолят солистый. Реакция проводилась неподходящей технологией при температуре 190 °C и давлении 45 атм. На основе работ Шульце и Кимминга известно, что это максимально токсическое вещество не образуется, если же вышеназванная реакция проводится при температуре ниже чем 153 °C. Поражениями были не только работающие при непосредственном производстве, но и рабочие по ремонту, а также научно-исследовательские работники и рабочие — земледелы, копающие канализационный канал.

У 6 из них началось заболевание острым солярным дерматитом, у остальных — медленным рассеянием комедонов и их переходом в кисты. Воспаления и гнои наблюдались в наиболее тяжелых случаях. Локализация и клинические признаки соответствовали тем, которые известны из современных литературных источников. У 17 больных, однако, acne chlorina начался необыкновенным способом — папулопустулами на конечностях. У 20 больных авторам удалось обнаружить локализацию в ахиллах, этот факт не был отмечен в современной литературе. Заболевание не проявляется на тех местах, где растут волосы, а у одного больного совершенно исчезло с тех местах лица и тела, где вырвали усы и бороду, причем в остальных местах заболевание продолжалось. Больные с нарушениями метаболизма порфирина имели типичную гиперпигментацию и гипертрихоз, причем один из них имел bullosis actinica et mechanica. Однако авторы обнаружили гипертрихоз и гиперпигментацию и у 19 пациентов, имеющих acne без лабораторных признаков порфирии.

В период перед заболеванием у больных не был найден предопределяющий момент. Латенция была различной. В некоторых случаях в после весьма короткий интервал наблюдались тяжелые повреждения как кожные, так и внутренние. В других случаях с длительным и массивным экспозиция вызвала только незначительные повреждения. Несмотря на это авторы уверены, что интенсивность заболевания в большей степени зависит от экспозиции, чем от предрасположения. У больных наблюдались субъективные ощущения в соматической, душевной и сенсуальной областях, у некоторых и потери веса. Два умерли от общей интоксикации, другие два от рака легких, взаимозависимые которого к профессиональной экспозиции, затем еще невозможно выразить. Раковое действие хлорированных углеводородов, на основе описанного производства затем еще нельзя доказать. В других работах авторы дают подробное описание внутренних и неврологических признаков своих больных.

Все больные находятся под постоянным наблюдением терапевта и невролога. После достижения максимума — обычно между 1-ым и 2-ым годом — заболевание постепенно уменьшается. У некоторых больных кожные заболевания и симптомы общей интоксикации продолжаются максимально 8 лет. Все применяемые методы оказались недостаточными. Улучшение наблюдалось при регулярной экспрессии желудка и при сауне. Все пострадавшие прошли лечение и им была возмещена убытки, в соответствии с существующими законами. Ки.

Ca. Derm., 48, 1973, č. 5, s. 306—317.

Jirásek L., Kalenský J., Kubec K.: Acne Chlorina and Porphyria Cutanea Tarda during the Manufacture of Herbicides

The authors submit after an evaluation of data reported in the literature results of the long-term follow up of 78 patients with an occupational disease caused by manufacture of herbicides — pentachlorphenol and derivatives of 2,4,5-trichlorophenoxyacetic acid. Of this number 78 subjects suffered from acne chlorina manifested in mild cases only by comedones and in severe cases by large cysts, abscesses and mutilating scars on the whole body. The varied clinical picture is demonstrated on photographs. Twelve of the patients had a hepatic lesion with symptoms of porphyria cutanea tarda and some patients had nervous and mental disorders.

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It was revealed that the skin and general affections were caused by 2,3,8,7-tetrachlorodibenzodioxin which is formed during alkaline hydrolysis of tetrachlorobenzene into sodium trichlorophenolate. The reaction was performed by an unsuitable procedure at a temperature of 190 °C and pressure of 45 atm. From the work of Schulz and Kimmig it is known that this highly toxic substances is not formed when the above reaction is carried out at a lower temperature of 153 °C. Not only workers engaged in production of herbicides were affected but also maintenance workers, research workers and workers digging the sewage channel.

In six the disease began as acute solar dermatitis, in all the remainder it started by a slow eruption of comedones and their development into cysts. Inflammations and scarring were found only in the most severe and extensive affections. The site and clinical manifestations corresponded to data reported in the literature. In 17 patients, however, acne chlorina started in a unusual way by papulopustulous eruptions on the extremities. In 20 patients the authors observed localization in the axillae which was not reported previously. The disease does not affect the hairy part of the skull and in one patient it disappeared even from those sites in the face and on the neck where he grew a beard, while the condition persisted at other sites. Patients with an impaired porphyria metabolism suffered from typical hyperpigmentation and hypertrichosis and only one of them from bullosis actinica et mechanica. Hypertrichosis and hyperpigmentation was observed also in 19 subjects with acne without laboratory signs of porphyria.

In the period prior to the disease no significant predisposing factors were noted. The latency differed. In some instances severe dermatological and internal damage developed after short exposure. In other instances apparently long and massive exposure caused only mild symptoms. Despite this the authors are convinced that the intensity of the disease depends more on exposure than predisposition. The patients had various complaints-somatic, mental and sexual and some of them had considerable weight losses. Two died from general intoxication, two from lung cancer but so far it is not possible to state reliably whether it was related to the occupational exposure. The cancerogenic effect of chlorinated hydrocarbons produced in the particular plant was not proved so far. The medical and neurological symptoms will be discussed by the authors in subsequent work.

All patients are being followed up in collaboration with specialists in internal medicine and a neurologist. After the peak has been reached — usually after 1—3 years duration — the disease recedes very slowly. In some patients, however, the manifestations and symptoms of general intoxication persist already for 8 years. All therapeutic methods proved little effective. A certain improvement was obtained by regular expression of comedones and sauna. In all patients secondary prevention was ensured, treatment and damages in accordance to legal provisions. O.

Ca. Derm., 48, 1973, č. 5, s. 308—317.

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SCHEDULE 84

I talked with V. E. Rowe of Dow, this morning. He stated the following:

1. Dow has 20 cases of chloracne. He did not tell me whether or not their plant was down.

2. They analyzed the trichlorophenoxyacetic acid we sent them and found 3 - 10 ppm of 3,4,7,8-trichlorodibenzodioxane. This, he states, he is sure is the chloracne agent, although there may be other impurities. At any rate, he is so concerned that they are having a management meeting to decide whether to use these five carloads of material we sent them.

3. He had definitely caused chloracne on rabbit ears with this material. When I tried to say that some of our people have not been able to cause it with other material, he just scoffed and said there was no doubt in his mind that he can tell chloracne by this rabbit ear test and that he has run about 500 tests on rabbit ears.

4. Dow has been receiving complaints from their customers (whether formulators or ultimate users, I don't know). He was not too clear on whose material it was and how much chloracne developed.

5. He had heard that "the FMS is looking into this problem and has contacted some of Dow's customers concerning the occurrence of chloracne."

6. Dow will have a management meeting today to decide whether or not they will accept this 2,4,5-T we sent them. He asked if we had any specifications on the presence of this particular contaminant & I told him we did not.

7. He wanted some sort of crash meeting with all the producers to try to reach some specification level in order to eliminate this particular contaminant before the FMS got into the act.

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EXHIBIT

Rowe-43 ID

3-21-84 J.L.F.

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[illegible] February 2-, 1965

[illegible] Rumer, [illegible] Burton, Irvan and Dunlap. All of the above was hashed over again. The only thing the Agricultural Division people could add was that they had no history of chloracne from any of their customers. They are going to call Amchem, who has bought about 1.2 million pounds of 2,4-D,to find out if they had any trouble. Rumer is to check Thompson Company to see if the PHS has been after them. They also stated that if such a meeting occurs Dr. Jacobs, who is a combination quality control/process man, would be their representative, along with someone from the Medical Department.

R. Emmet Kelly, M. D.

REK/ln

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KEISTER V. DOW

KEISTER V. DOW
EXHIBITS #1-49

DEBORAH A. BARSOTTI, Ph.D.

I. QUALIFICATIONS

Dr. Barsotti is an Assistant Professor of Tocicology in the Department of Pharmacology and Toxicology of the Philadelphia College of Pharmacy and Science in Philadelphia, Pennsylvania. Her curriculum vitae is attached.

II. SUBJECT MATTER

Dr. Barsotti is a toxicologist who has done substantial personal investigative work with halogenated aromatic hydrocarbons. This category of chemicals includes various biphenyls and dioxins, and specifically includes tetrachlorodibenzo-p-dioxin (TCDD). She will testify as to the relative chemical stability and lipophilicity of these chemicals as well as their resistance to degradation, and will testify that these qualities are what account for the perceived and documented bioaccumulation of the chemicals in the human system. Dr. Barsotti will emphasize the difference in results between chronic and acute exposures to these chemicals, testifying as to the varying types of responses to acute and chronic exposures.

Dr. Barsotti will discuss her own work as well as review the literature in these areas, noting differences in routes of

administration, and in the species and genetic strain of the animals involved. She will explain these variations and where they permit extrapolation of results from animals to humans. In particular, Dr. Barsotti will also testify as to the similarities in response to these chemicals by rhesus monkeys and by humans, and will explain how the similarities permit extrapolation in this instance.

Dr. Barsotti will also discuss reproductive toxicity, teratology and post-natal toxicity, including instances where no gross or clinical signs of maternal toxicity could be detected.

III. SCIENTIFIC PRINCIPLES

See General Statement of Scientific Principles and Assumed Facts attached.

IV. SUBSTANCE OF OPINIONS

A. That dioxins are toxic, and in particular TCDD is exceptionally toxic.

B. That dioxins bioaccumulate because of their chemical stability, lepophilicity, and resistance to degradation.

C. That chronic low level exposure to dioxins is significantly toxic.

D. That TCDD accumulates and persists in the lipid stores.

E. That TCDD, in chronic doses, is toxic, is particularly toxic to reproductive system, is teratogenic, and is fetotoxic.

F. There are ample scientific bases, for extrapolating animal experimental data to man, in the case of TCDD, for example, as demonstrated by Dr. Barsotti's work on rhesus monkeys.

V. GROUNDS FOR EACH OPINION

A. General

Review of applicable medical and scientific literature; education and training; personal research. The work on the chronic toxicity of HAHs in rhesus monkeys has culminated in numerous publications as presented in Dr. Barsotti's curriculum vitae. In addition, she has been invited to speak or present her work in various atmospheres including community meetings and scientific symposia.

B. Specific

1. With respect to opinion IV A, see generally references attached.

2. With respect to Opinion IV B, note the following:

In the course of Dr. Barsotti's graduate and post-graduate studies, she has had the opportunity to conduct research and evaluate others' work on members of the class of chemicals called halogenated aromatic hydrocarbons (HAHs). She will discuss the fact that this class of chemicals includes the chlorinated dibenzo-p-dioxins and the halogenated biphenyls (polychlorinated and polybrominated biphenyls) and that HAHs are similar in chemical structure, in the pattern of toxic responses that they produce and in the mechanism by which they are believed to act (Allen et al., 1979; Poland and Knutson, 1982). Although individual HAHs vary in potency, their chemical stability and lipophilicity, as well as their resistance to degradation results in bioaccumulation (Isensee and Jones, 1975; Meselson et al., 1978; Marinucci and Bartha, 1982; Mes et al., 1982).

3. With respect to opinion IV C, note the following:

Thesis work performed by Dr. Barsotti at the University of Wisconsin with polychlorinated biphenyls indicated that evaluation of the toxic effects of HAHs in the Rhesus monkeys was valuable to the understanding of the potential hazards of HAHs in the environment (i.e. chronic exposure). In addition, this work indicated, unlike rodent models, that there were similarities in the response of humans and rhesus monkeys to these chemical toxicants (Barsotti, 1980). Like man, the rhesus monkey is genetically diverse and responded to PCB exposure on an individual basis.

Dr. Barsotti will contrast the acute toxicity that one observes in accidental or intentional ingestion of toxic or lethal doses of chemicals with the effects of chronic or lower levels of exposure to chemicals such as HAHs.

4. With respect to Opinion IV D, note the following:

Dr. Barsotti will comment on the state of the literature on the fate of TCDD once in the body, i.e. TCDD metabolism (Vinopal and Cassida, 1973; Ramsey et al., 1979; Schlatter, 1979). Efforts to identify metabolites in tissues and excreta from various studies have been unsuccessful. Recent advances in detection methodology indicate elevated levels of TCDD in Vietnam veterans and other exposed persons. Generally, however, the data indicate that if metabolism does occur the rate is slow (Guenthner et al., 1979). The ramification of this finding is that the body would be incapable of or slow in ridding itself of TCDD and TCDD would accumulate and persist in the lipid stores. In Dr. Barsotti's work, as well as others', this appears to be the case with rhesus monkeys and man (Montagna et al., 1979; Van Miller, 1981; McNulty et al., 1983).

5. With respect to opinion IV E, note the following:

Not only did the rhesus monkey appear a good model for HAH toxicity but established, as had been in other animal species, that the reproductive system was extremely sensitive to PCBs at intake levels below that required for the manifestation of toxic signs in the adult (Barsotti et al., 1976). In addition to PCB induced reproductive toxicity, PCBs were found to be fetotoxic

three months in animals receiving TCDD in the diets at levels of 500 parts per trillion (ppt) and in animals consuming diets containing 50 ppt TCDD these same symptoms occurred after consuming the diets for 30 months (Allen et al., 1977; Schantz et al., 1979). This indicates cumulative toxicity associated with ingestion of TCDD. Thus, there is evidence for bioaccumulation and associated chronic toxicity at lower doses than that required for acute toxicity in rhesus monkeys and presumably man (Pazderova et al, 1981; Ideo et al., 1982.)

6. With respect to Opinion IV F, see generally references attached and V A above.

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UNITED STATES PATENT OFFICE

2,509,245

PREPARATION OF 2,4,5-TRICHLOROPHENOL

Edward Joseph Nikawitz, Passaic, and William S. Gump, Upper Montclair, N. J., assignors to The Givaudan Corporation, a corporation of New Jersey

No Drawing. Application March 20, 1947, Serial No. 736,118

5 Claims. (Cl. 260—623)

1

This invention relates to a process for preparing 2,4,5-trichloro phenol, and more especially to a process wherein 1,2,4,5-tetrachloro benzene is subjected to alkaline hydrolysis in the presence of ethylene- or propylene glycol (propanediol-1,2).

2,4,5-trichloro phenol has been prepared from 1,2,4,5-tetrachloro benzene by hydrolyzing the latter with alkali in the presence of methyl alcohol, the process being conducted under considerable pressure, of the order of 600–800 pounds per square inch. Special pressure equipment is required for conducting such a process. Moreover, appreciable amounts of the methyl ether of 2,4,5-trichloro phenol form when methyl alcohol is employed; and the formation of the ether is undesirable as it decreases the yield of the desired free phenol.

Our present invention overcomes the foregoing disadvantages and provides a process for making 2,4,5-trichloro phenol from 1,2,4,5-tetrachloro benzene which can be conducted with cheaper and simpler equipment than is required by the prior art process, and which does not result in the formation of any appreciable amount of ether.

In general, our process may be conducted by dissolving an alkali metal hydroxide, such as sodium hydroxide potassium hydroxide and lithium hydroxide, in ethylene glycol or propylene glycol, or a mixture thereof, at elevated temperatures while stirring the contents. The tetrachloro benzene is then added and the mixture is heated for a few hours, normally 3–4 hours being sufficient. The end point of the reaction can be determined easily by taking a sample of the reaction mixture and diluting it with water. If the sample is water soluble or practically entirely soluble in the water, the reaction may be considered to have been completed. The desired phenol may be isolated in accordance with known procedures. For example, the reaction mixture may be cooled after the test as above shows substantial completion of the reaction, and then acidified with a mineral acid such as hydrochloric acid. The precipitated alkali metal chloride is filtered off. The filtrate is poured into water, causing the 2,4,5-trichloro phenol to precipitate. The phenol is extracted with benzene and the benzene extract is distilled to remove the benzene and yield the phenol. The aqueous layer remaining after the benzene extraction is fractionally distilled to remove the glycol employed.

The proportions of the ingredients used may

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be varied. The alkali metal hydroxide is used in amounts equivalent to at least 2 mols of hydroxide per mol of tetrachloro benzene. 2–3 mols of hydroxide per mol of tetrachloro benzene gives excellent results. Higher amounts of hydroxide may be employed, but are unnecessary.

With regard to the amount of glycol which should be employed in our process, we find that excellent results are obtained when about 750 grams of the glycol per 216 grams (1 mol) of the tetrachloro benzene are used. Larger amounts of glycol may be used, but in such cases no advantageous results follow. Amounts less than 450 grams of glycol per 216 grams of tetrachloro benzene are not recommended, as yield and quality of the desired phenol are adversely affected.

The temperature range at which the hydrolysis may be effected is between about 160° C. and 200° C., the preferred range being between about 170° C. and 180° C. Higher temperatures are obtainable when propylene glycol is employed than is the case when ethylene glycol is employed.

A special advantage of this process is that it can be conducted at atmospheric pressure, under reflux. However, if desired, the contents may be heated in a closed system, whereby a slight pressure is built up, amounting however to not more than 15 to 20 pounds per square inch, and not necessitating the use of any special pressure equipment in the plant.

The invention is illustrated by the following examples without however limiting the same to them.

Example I

60 grams of sodium hydroxide flakes (95% NaOH) were dissolved in 500 grams of ethylene glycol in a 2 liter three-necked flask provided with stirrer and an air condenser. The contents were heated to 150° C.–160° C., this step requiring about 30 minutes. 144 grams of 1,2,4,5-tetrachloro benzene were rapidly added to the solution, and the mixture was heated to 170° C.–180° C. (inside temperature), and maintained at that temperature range for 4 hours. 10 grams of tetrachloro benzene sublimed in the air condenser and were recovered. A sample of the reaction mixture gave a clear solution when dissolved in 10 times its weight of water.

The reaction mixture was allowed to cool; dry hydrogen chloride was passed into it until it became acid to litmus. The slight excess of hydrogen chloride was neutralized by the addition of a small amount of sodium bicarbonate. After cooling again to about 20° C., the salt was filtered

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This invention relates to a process for preparing 2,4,5-trichloro phenol, and more especially to a process wherein 1,2,4,5-tetrachloro benzene is subjected to alkaline hydrolysis in the presence of ethylene- or propylene glycol (propanediol-1,2).

2,4,5-trichloro phenol has been prepared from 1,2,4,5-tetrachloro benzene by hydrolyzing the latter with alkali in the presence of methyl alcohol, the process being conducted under considerable pressure, of the order of 600–800 pounds per square inch. Special pressure equipment is required for conducting such a process. Moreover, appreciable amounts of the methyl ether of 2,4,5-trichloro phenol form when methyl alcohol is employed; and the formation of the ether is undesirable as it decreases the yield of the desired free phenol.

Our present invention overcomes the foregoing disadvantages and provides a process for making 2,4,5-trichloro phenol from 1,2,4,5-tetrachloro benzene which can be conducted with cheaper and simpler equipment than is required by the prior art process, and which does not result in the formation of any appreciable amount of ether.

In general, our process may be conducted by dissolving an alkali metal hydroxide, such as sodium hydroxide, potassium hydroxide and lithium hydroxide, in ethylene glycol or propylene glycol, or a mixture thereof, at elevated temperatures while stirring the contents. The tetrachloro benzene is then added and the mixture is heated for a few hours, normally 3–4 hours being sufficient. The end point of the reaction can be determined easily by taking a sample of the reaction mixture and diluting it with water. If the sample is water soluble or practically entirely soluble in the water, the reaction may be considered to have been completed. The desired phenol may be isolated in accordance with known procedures. For example, the reaction mixture may be cooled after the test as above shows substantial completion of the reaction, and then acidified with a mineral acid such as hydrochloric acid. The precipitated alkali metal chloride is filtered off. The filtrate is poured into water, causing the 2,4,5-trichloro phenol to precipitate. The phenol is extracted with benzene and the benzene extract is distilled to remove the benzene and yield the phenol. The aqueous layer remaining after the benzene extraction is fractionally distilled to remove the glycol employed.

The proportions of the ingredients used may

2

be varied. The alkali metal hydroxide is used in amounts equivalent to at least 2 mols of hydroxide per mol of tetrachloro benzene. 2–3 mols of hydroxide per mol of tetrachloro benzene gives excellent results. Higher amounts of hydroxide may be employed, but are unnecessary.

With regard to the amount of glycol which should be employed in our process, we find that excellent results are obtained when about 750 grams of the glycol per 216 grams (1 mol) of the tetrachloro benzene are used. Larger amounts of glycol may be used, but in such cases no advantageous results follow. Amounts less than 450 grams of glycol per 216 grams of tetrachloro benzene are not recommended, as yield and quality of the desired phenol are adversely affected.

The temperature range at which the hydrolysis may be effected is between about 160° C. and 200° C., the preferred range being between about 170° C. and 180° C. Higher temperatures are obtainable when propylene glycol is employed than is the case when ethylene glycol is employed.

A special advantage of this process is that it can be conducted at atmospheric pressure, under reflux. However, if desired, the contents may be heated in a closed system, whereby a slight pressure is built up, amounting however to not more than 15 to 20 pounds per square inch, and not necessitating the use of any special pressure equipment in the plant.

The invention is illustrated by the following examples without however limiting the same to them.

Example I

60 grams of sodium hydroxide flakes (95% NaOH) were dissolved in 500 grams of ethylene glycol in a 2 liter three-necked flask provided with stirrer and an air condenser. The contents were heated to 150° C.–160° C., this step requiring about 30 minutes. 144 grams of 1,2,4,5-tetrachloro benzene were rapidly added to the solution, and the mixture was heated to 170° C.–180° C. (inside temperature), and maintained at that temperature range for 4 hours. 10 grams of tetrachloro benzene sublimed in the air condenser and were recovered. A sample of the reaction mixture gave a clear solution when dissolved in 10 times its weight of water.

The reaction mixture was allowed to cool; dry hydrogen chloride was passed into it until it became acid to litmus. The slight excess of hydrogen chloride was neutralized by the addition of a small amount of sodium bicarbonate. After cooling again to about 20° C., the salt was filtered

5938

by suction and the salt cake was washed with 50 cc. of isopropyl alcohol. 600 cc. of water were added to the filtrate resulting in a bottom layer of precipitated trichloro phenol and a top layer of dilute ethylene glycol. The entire mixture was extracted with 400 cc. of benzene, then with 100 cc. of benzene and finally with 80 cc. of benzene.

The combined benzene extracts were shaken with 200 cc. of water and the water layer was separated and added to the dilute ethylene glycol. The washed combined benzene extracts were dried by means of anhydrous sodium sulfate, filtered, and distilled. After removal of the benzene, the residue was distilled at a pressure of 4 mm. of mercury. 106 grams of 2,4,5-trichloro phenol, boiling at 101° C.–105° C., and having a congealing point of 63.3° C. (uncorrected), were obtained.

The ethylene glycol can be recovered by distillation of the aforementioned dilute ethylene glycol. The water and isopropyl alcohol were removed in a fractionating still at a pressure of 90 mm. of mercury, the temperature being carried up to 50° C. The ethylene glycol was then distilled under high vacuum (3 mm.), 232 grams of the glycol boiling at 80° C. being recovered. In order to remove practically all of the ethylene glycol from the small amount of salt remaining in the distilling flask, the temperature was raised so that some glycol, boiling from 80° C. to 120° C., was obtained.

Example II

72 grams of 1,2,4,5-tetrachloro benzene were stirred and heated to 190–200° C. with a solution of 30 grams of sodium hydroxide in 250 grams of propylene glycol, the heat treatment being conducted for 6 hours. 24 grams of concentrated sulfuric acid (93% H_2SO_4) were added to the reaction contents after they were cooled to room temperature (about 25° C.). The entire contents were poured into 1000 cc. of water. The solid material was then filtered and washed with 500 cc. of water and finally dissolved in 200 cc. of benzene. The benzene solution was dried with anhydrous sodium sulfate and then filtered.

After removal of the benzene by distillation, the residue was distilled under a high vacuum (5 mm.), 45 grams of 2,4,5-trichloro phenol being obtained thereby.

The foregoing illustrates the practice of this invention, which however, is not to be limited thereby but is to be construed as broadly as permissible in view of the prior art and limited solely by the appended claims.

We claim:

1. The process for preparing 2,4,5-trichlorophenol, which comprises heating at 160°–200° C. in the following proportions: 1 gram molecular weight of 1,2,4,5-tetrachloro benzene and at least 2 gram molecular weights of an alkali metal hydroxide in the presence of at least 450 grams of at least one material from the group consisting of ethylene glycol and propylene glycol, the reaction being conducted under a pressure within the range of that of the atmosphere up to 20 pounds per square inch and for a time sufficient to substantially complete the conversion into 2,4,5-trichlorophenol.

2. The process for preparing 2,4,5-trichlorophenol, which comprises heating at 160°–200° C. in the following proportions: 1 gram molecular weight of 1,2,4,5-tetrachloro benzene and at least 2 gram molecular weights of an alkali metal hydroxide in the presence of at least 450 grams of ethylene glycol, the reaction being conducted under a pressure within the range of that of the atmosphere up to 20 pounds per square inch and for a time sufficient to substantially complete the conversion into 2,4,5-trichlorophenol.

3. The process for preparing 2,4,5-trichlorophenol, which comprises heating at 160°–200° C. in the following proportions: 1 gram molecular weight of 1,2,4,5-tetrachloro benzene and at least 2 gram molecular weights of sodium hydroxide in the presence of at least 450 grams of ethylene glycol, the reaction being conducted under a pressure within the range of that of the atmosphere up to 20 pounds per square inch and for a time sufficient to substantially complete the conversion into 2,4,5-trichlorophenol.

4. The process for preparing 2,4,5-trichlorophenol, which comprises heating at 170°–180° C. in the following proportions: 1 gram molecular weight of 1,2,4,5-tetrachloro benzene and 2–3 gram molecular weights of sodium hydroxide in the presence of 750 grams of ethylene glycol, the reaction being conducted under atmospheric pressure and for a time sufficient to substantially complete the conversion into 2,4,5-trichlorophenol.

5. The process for preparing 2,4,5-trichlorophenol, which comprises heating at 170°–180° C. in the following proportions: 1 gram molecular weight of 1,2,4,5-tetrachloro benzene and 2–3 gram molecular weights of sodium hydroxide in the presence of 750 grams of propylene glycol, the reaction being conducted under atmospheric pressure and for a time sufficient to substantially complete the conversion into 2,4,5-trichlorophenol.

EDWARD JOSEPH NIKAWITZ.
WILLIAM S. GUMP.

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United States Patent Office 2,799,713
Patented July 16, 1957

2,799,713

METHOD OF MAKING TRICHLOROPHENOLS FROM TETRACHLOROBENZENES

Alexander H. Widiger, Jr., Midland, Mike L. Aaron, Hemlock, and Gordon F. Dugan, Beaverton, Mich., assignors to The Dow Chemical Company, Midland, Mich., a corporation of Delaware

No Drawing. Application January 24, 1955, Serial No. 483,798

5 Claims. (Cl. 260—623)

This invention concerns an improved process for hydrolyzing tetrachlorobenzenes to produce trichlorophenols. It relates more particularly to improvements in a process for carrying out the hydrolysis of a tetrachlorobenzene to produce a trichlorophenol.

United States Patent No. 2,615,923 forms trichlorophenols by reacting tetrachlorobenzenes with an alkali metal hydroxide dissolved in, or mixed with, methyl alcohol at elevated temperatures and pressures. U. S. Patent No. 2,509,245 makes 2,4,5-trichlorophenol by heating 1,2,4,5-tetrachlorobenzene at temperatures between 170° and 190° C. at atmospheric to 20 pounds pressure with an alkali metal hydroxide dissolved in, or mixed with ethylene glycol or propylene glycol.

There are several objections to the methods heretofore proposed for the preparation of trichlorophenols from tetrachlorobenzenes particularly when applied to the alkaline hydrolysis of tetrachlorobenzenes to produce trichlorophenols on a commercial scale. The principal disadvantage is that of employing an organic liquid or solvent in the hydrolysis reaction. The employment of an organic liquid in the hydrolysis results in the formation of byproduct ethers and increases the problems of separation and recovery of the final product.

When methyl alcohol is employed in the hydrolysis considerable quantities of the alcohol are consumed in forming ethers, e. g. dimethylether, or methyl ether of trichlorophenol, and the alcohol must be replaced by new supplies. The employment of a glycol in the reaction increases the problems of separation and recovery of the final product. Also, the employment of an organic liquid or solvent in the hydrolysis takes up valuable reactor space and lowers the capacity for reactants of a given reaction vessel, is expensive and adds to the cost of the final product.

It has now been found that a tetrachlorobenzene having the empirical formula $C_6H_2Cl_4$, e. g. 1,2,4,5-tetrachlorobenzene, or a mixture of isomeric tetrachlorobenzenes, can readily be hydrolyzed, whereby a single chlorine atom is replaced by a hydroxyl group or the corresponding metaloxy group to produce trichlorophenols or salts thereof in good yield, by heating the tetrachlorobenzene in admixture with an alkali metal hydroxide and water at elevated temperatures and pressures as hereinafter described.

More specifically, it has been discovered that the alkaline hydrolysis of tetrachlorobenzenes to produce trichlorophenols in good yield can readily be carried out under the following set of reaction conditions: (*a*) carrying out of the hydrolysis reaction at temperatures between 225° and 300° C., preferably from 240° to 280° C.; (*b*) employing the alkali metal hydroxide in amount corresponding to from 1.8 to 4.5, preferably from 2 to 4, gram molecular proportions of the alkali metal hydroxide per gram molecular equivalent proportion of the tetrachlorobenzene and in admixture with sufficient water to form an aqueous solution containing from 2 to 10, preferably from 2 to 8, percent by weight of the alkali metal hydroxide; and (*c*) carrying out of the hydrolysis under a pressure at least as high as the autogenous pressure of the mixture of materials. The hydrolysis is carried out in a reaction time of not more than 120 minutes, preferably from about 6 to 90 minutes.

The invention concerns a process for hydrolyzing tetrachlorobenzenes which involves employing each of the reaction conditions set forth in items (*a*), (*b*) and (*c*) just mentioned and results in good yields of trichlorophenols. The invention utilizes a readily available, inexpensive, non-flammable reaction medium, and permits carrying out of the alkaline hydrolysis of tetrachlorobenzenes to produce trichlorophenols in a manner suitable for the manufacture of trichlorophenols, batchwise, or in continuous manner, on an economically commercial scale.

Sodium hydroxide is the preferred alkali metal hydroxide to be employed in the hydrolysis. Other alkali metal hydroxides such as potassium hydroxide, or lithium hydroxide, or mixtures of any two or more alkali metal hydroxides can be used.

The alkali metal hydroxide is employed in amount corresponding to from 1.8 to 4.5, preferably from 2 to 4 gram molecular proportions per gram molecular equivalent proportion of the tetrachlorobenzene employed, and in admixture with sufficient water to form an aqueous solution of the alkali metal hydroxide in from 2 to 10, preferably from 2 to 8, percent by weight concentration.

The hydrolysis is carried out with agitation at temperatures between 225° and 300° C., preferably from 240° to 280° C., and under a pressure at least as high as the autogenous pressure of the mixture of the materials. Pressures of from about 400 to 1500 pounds per square inch are satisfactory and pressures of from 400 to 3000 pounds per square inch gauge, or higher can be used.

The hydrolysis proceeds readily under the aforementioned reaction conditions to produce the trichlorophenols or salts thereof in appreciable yield in a reaction time of from about 6 to 120 minutes. The rate at which the reaction occurs is dependent in part upon the concentration of the alkali metal hydroxide in the aqueous starting solution and the reaction temperature employed, within the ranges here specified. The degree of hydrolysis is dependent upon the reaction time, under otherwise similar reaction conditions. At the higher reaction temperatures, e. g. 250° to 300° C., herein specified, the hydrolysis reaction proceeds to completion at a faster rate and in a shorter time than at lower temperatures. The mixture of materials should not be heated at the reaction temperatures herein specified for prolonged periods of time such as to result in the removal of more than one chlorine atom from a molecule of the tetrachlorobenzene starting material, or result in deterioration of the trichlorophenol product.

In practice, the tetrachlorobenzene, the alkali metal hydroxide and water, or an aqueous solution of the alkali metal hydroxide, are placed in a suitable pressure resistant vessel, e. g. an iron, steel, or stainless steel autoclave, in the desired proportions. The mixture is agitated and heated at reaction temperatures between 225° and 300° C. under at least the autogenous pressure of the mixture of materials for a reaction time of from about 6 to 120 minutes, then cooled. The hydrolysis mixture is removed from the reactor. In the instance where the tetrachlorobenzene starting material, e. g. 1,2,4,5-tetrachlorobenzene, is a crystalline material at ordinary temperatures the unreacted tetrachlorobenzene is usually recovered by filtering the hydrolysis mixture. The filtrate, or aqueous hydrolysis liquor containing the trichlorophenol in the form

5941

of its alkali metal salt, is thereafter acidified with an acid such as sulfuric acid, or hydrochloric acid, preferably the latter. The trichlorophenol is separated from the aqueous liquor in usual ways, e. g. by decanting or filtering if the product is a solid, or by extraction of the aqueous acidic liquor with a water-immiscible solvent such as benzene, toluene, or chlorobenzene to dissolve the trichlorophenol. The trichlorophenol is recovered from the solvent in usual ways, e. g. by distillation.

The hydrolysis of the tetrachlorobenzene can be carried out in continuous manner by feeding the tetrachlorobenzene and a solution of the alkali metal hydroxide and water in the desired proportions to a reaction zone, e. g. a tubular reactor or other suitable pressure resistant vessel, wherein the materials are agitated and heated in admixture with one another under pressures at least as high as the autogenous pressure and at temperatures and for reaction times as previously described, and thereafter cooling and discharging the hydrolyzed mixture from the reaction zone, after which the trichlorophenol product is recovered in usual ways.

The following examples illustrate ways in which the principle of the invention has been applied but are not to be construed as limiting its scope.

EXAMPLE 1

A charge of 113.4 grams (0.525 mole) of 1,2,4,5-tetrachlorobenzene having a freezing point of 138° C., 42 grams (1.05 moles) of sodium hydroxide and 900 cc. of water, was placed in an iron bomb. The mixture was agitated and heated at a temperature of 250° C. under the autogenous pressure of the mixture of materials for a period of 20 minutes, then cooled. The bomb was opened and the contents removed. The mixture was filtered to recover the unreacted 1,2,4,5-tetrachlorobenzene, which was washed with water and dried. There was obtained 38.3 grams of unreacted 1,2,4,5-tetrachlorobenzene. The conversion was 66 percent, based on the tetrachlorobenzene starting material. The filtrate, i. e. the aqueous hydrolysis liquor, was acidified with dilute hydrochloric acid. The acidic mixture was extracted three times, each with a 100 cc. portion of benzene, and the benzene solutions combined. The solution was distilled to separate the components. There was obtained 56.3 grams (0.28 mole) of 2,4,5-trichlorophenol having a freezing point of 63.5° C. and 1.8 grams of residue. The yield of 2,4,5-trichlorophenol product is 80.5 percent, based on the 1,2,4,5-tetrachlorobenzene consumed in the reaction.

EXAMPLE 2

In each of a series of experiments, a charge of 37.8 grams of 1,2,4,5-tetrachlorobenzene (freezing point 131° C.), 14 grams (0.35 mole) of sodium hydroxide and 300 cc. of water was placed in an iron bomb. The mixture was agitated and heated at a temperature and for a reaction time stated in the following table under the autogenous pressure of the materials, then cooled. The reacted materials were removed from the bomb. The mixture was filtered to separate the unreacted tetrachlorobenzene. The filtrate was acidified with aqueous hydrochloric acid and the acidic mixture extracted with benzene to separate the trichlorophenol product from the aqueous liquor. The product was recovered by heating the benzene solution to distill and separate the benzene from the 2,4,5-trichlorophenol. The product was obtained as residue after distillation of the benzene. Table I identifies the experiments and gives the temperature and the time in minutes for which the hydrolysis reaction was carried out. The table gives the percent conversion based on the tetrachlorobenzene starting material. The table also gives the weight in grams of the unreacted 1,2,4,5-tetrachlorobenzene and the weight of the 2,4,5-trichlorophenol product obtained and the yield of said product, based on the 1,2,4,5-tetrachlorobenzene consumed in the reaction.

Table I

Run No.	Reaction Conditions		Conversion, Percent	Products		
	Time, Min.	Temp., °C.		1,2,4,5-Tetra-Chloro-Benzene, Gms.	2,4,5-Trichlorophenol	
					Gms.	Yield, Percent
1	120	225	64	12.6	18.2	82
2	60	240	73	10.4	19.2	76.8
3	30	260	80	7.5	19.8	68.5
4	10	300	81	7.6	19.4	70.5

EXAMPLE 3

In each of a series of experiments, a charge of 1,2,4,5-tetrachlorobenzene similar to that employed in Example 2, and sodium hydroxide in proportions corresponding to one gram molecular proportion of the tetrachlorobenzene and two gram molecular equivalent proportions of the sodium hydroxide, and sufficient water to form an aqueous solution of the sodium hydroxide in concentration as stated in the following table, was placed in an iron bomb. The mixture was agitated and heated at a temperature of 275° C. under the autogenous pressure of the materials for a period of 10 minutes, then cooled. The trichlorophenol was recovered from the reacted mixture employing procedures similar to those described in Example 2. Table II identifies the experiments and gives the concentration of the sodium hydroxide in percent by weight in the aqueous starting solution. The table gives the percent conversion, the weight in grams of the recovered 1,2,4,5-tetrachlorobenzene, the weight in grams of 2,4,5-trichlorophenol obtained and the percent yield of said product, based on the 1,2,4,5-tetrachlorobenzene consumed in the reaction.

Table II

Run No.	Hydrolysis Solution	Conversion, Percent	Products		
	Concentration of Sodium Hydroxide in Water, Percent		1,2,4,5-Tetra-Chloro-Benzene, Gms.	2,4,5-Trichlorophenol	
				Gms.	Yield, Percent
1	2	90	2.0	12.9	88.4
2	5.0	78	9.0	20.4	78
3	10	55	40.6	22.9	53.7

EXAMPLE 4

A charge of 43.2 grams of crude tetrachlorobenzenes consisting of 75 percent by weight of 1,2,3,4-tetrachlorobenzene, 18 percent of 1,2,4,5-tetrachlorobenzene and 7 percent of 1,2,4-trichlorobenzene was placed in an iron bomb. A charge of 16 grams of sodium hydroxide and 304 cc. of water were added. The mixture was agitated and heated at a temperature of 250° C. under the autogenous pressure of the materials for a period of 20 minutes, then cooled. The bomb was opened and the contents removed. The reacted materials were extracted with benzene. The aqueous and organic layers were separated. The organic layer was distilled. There was obtained 16.4 grams of unreacted tetrachlorobenzenes. The aqueous hydrolysis liquor was made acidic with hydrochloric acid, then extracted with benzene. The benzene solution was heated to distill the benzene. There was obtained as residue or product 22.8 grams of trichlorophenols. The product was analyzed and found to consist of 65 percent by weight of 2,3,6-trichlorophenol, 18 percent of 2,4,5-trichlorophenol, and 17 percent of 2,3,4-trichlorophenol.

EXAMPLE 5

In each of a series of experiments, a charge of 1,2,4,5-

tetrachlorobenzene (freezing point 131° C.) and sodium hydroxide in proportion as stated in the following table, together with sufficient water to form an aqueous 5 weight percent solution of the sodium hydroxide, was placed in an iron bomb. The mixture was agitated and heated at a temperature of 250° C. for a period of 20 minutes, then cooled. The reacted materials were removed from the bomb. The products were recovered employing procedures similar to those described in Example 2. Table III identifies the experiments and gives the gram molecular proportions of 1,2,4,5-tetrachlorobenzene and sodium hydroxide employed in the hydrolysis reaction. The table gives the time and temperature conditions under which the hydrolysis was carried out. The table also gives the percent conversion, based on the tetrachlorobenzene starting material, the yield of 2,4,5-trichlorophenol obtained, based on the 1,2,4,5-tetrachlorobenzene consumed in the reaction, and the freezing point of the trichlorophenol.

Table III

Run No.	1,2,4,5-Chloro-Benzene, Mole	Sodium Hydroxide, Moles	Reaction Conditions		Conversion, Percent	2,4,5-Trichlorophenol	
			Time, Min.	Temp., °C.		Yield, Percent	Freezing Point, °C.
1	1	2	20	250	69	80	55.7
2	1	2.5	20	250	81	79	56.8
3	1	3.0	20	250	84	80	55.1
4	1	4.0	20	250	99	79	52.8

We claim:

1. In a process for making a trichlorophenol by the alkaline hydrolysis of a tetrachlorobenzene, the step which consists in hydrolyzing a tetrachlorobenzene by heating a mixture of one gram molecular proportion of a tetrachlorobenzene, from 1.8 to 4.5 gram molecular equivalent proportions of an alkali metal hydroxide and sufficient water to form an aqueous 2 to 10 weight percent solution of the alkali metal hydroxide, at reaction temperatures between 225° C. and 300° C. under at least the autogenous pressure of the mixture.

2. A process as claimed in claim 1, wherein the alkali metal hydroxide is sodium hydroxide.

3. In a process for making a trichlorophenol by the alkaline hydrolysis of a tetrachlorobenzene, the steps which consist in hydrolyzing 1,2,4,5-tetrachlorobenzene by heating a mixture of one gram molecular proportion of the 1,2,4,5-tetrachlorobenzene, from 1.8 to 4.5 gram molecular equivalent proportions of sodium hydroxide and sufficient water to form an aqueous 2 to 10 weight percent solution of the sodium hydroxide, at reaction temperatures between 225° and 300° C. under at least the autogenous pressure of the mixture of materials for a reaction time of from 6 to 120 minutes, then cooling the mixture and recovering 2,4,5-trichlorophenol from the hydrolyzed mixture.

4. A process as claimed in claim 3, wherein the sodium hydroxide is employed in amount corresponding to from 2 to 4 gram molecular proportions per gram molecular equivalent proportion of the tetrachlorobenzene starting material.

5. In a process for making a trichlorophenol by the alkaline hydrolysis of a tetrachlorobenzene, the steps which consist in hydrolyzing a tetrachlorobenzene by continuously feeding a tetrachlorobenzene and sodium hydroxide dissolved in sufficient water to form an aqueous 2 to 10 weight percent solution of the sodium hydroxide, in proportions corresponding to from 1.8 to 4.5 gram molecular proportions of the sodium hydroxide per gram molecular equivalent proportion of the tetrachlorobenzene, to a reaction zone wherein the materials are heated in admixture with one another at temperatures between 225° and 300° C. under at least the autogenous pressure of the mixture of materials for a reaction time of from 6 to 120 minutes, continuously withdrawing the reacted mixture from said reaction zone, and separating the trichlorophenol from the hydrolyzed mixture.

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(Corresponding Great Britain, 718,779, Nov. 17, 1954)

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TED IN U.S.A.



ERCULES POWDER COMPANY

August 2, 1962

JACKSONVILLE, ARKANSAS
OPERATIONS BOOKLET

This booklet gives information on the chemistry, properties, equipment, method of manufacture etc. for the various items produced at the Jacksonville, Arkansas plant. It is a summary of the operations as they currently exist.

It should be noted that Jacksonville personnel helped considerably in compiling the data presented in the booklet.

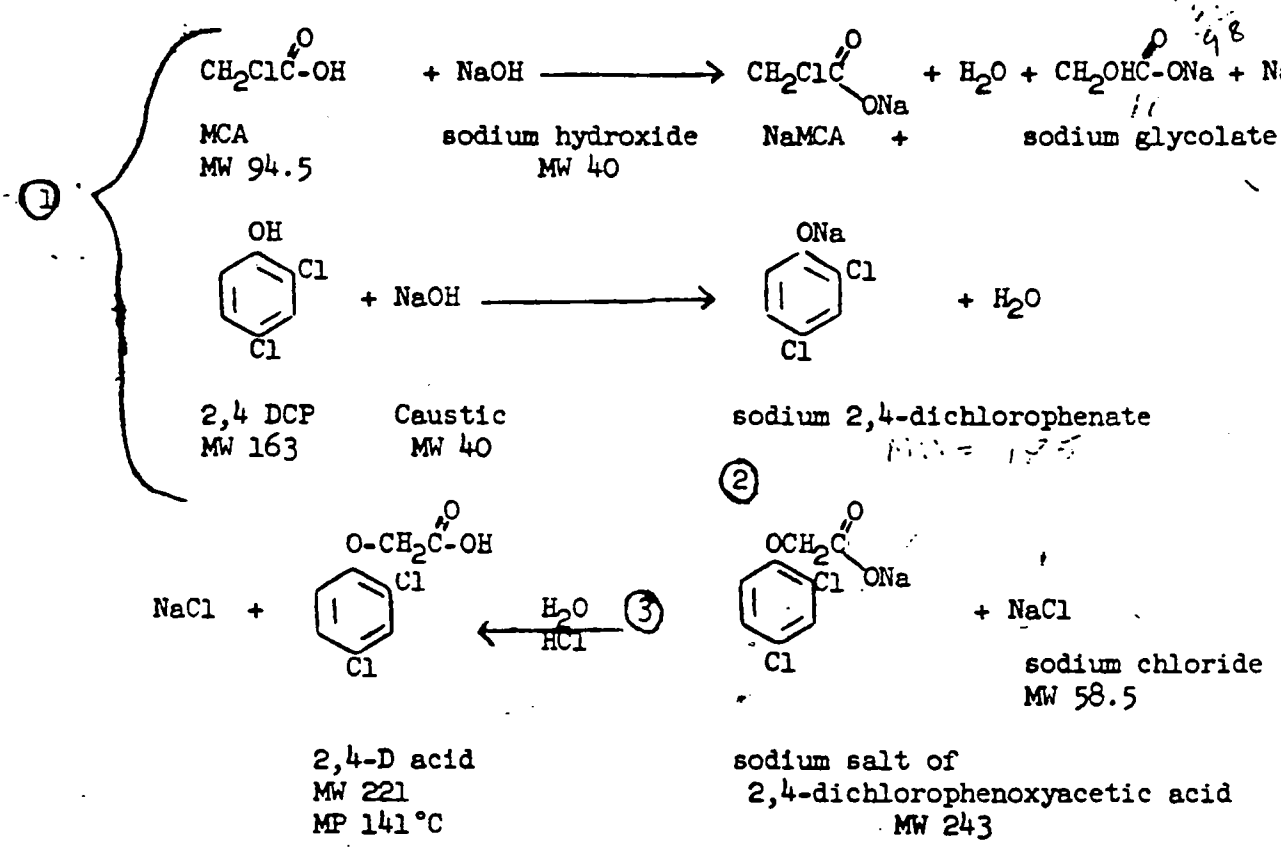
H. W. Gilder

5945

2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

CHEMISTRY:

2,4-dichlorophenoxyacetic acid is prepared by coupling monochloracetic acid with dichlorophenol as follows:



This is essentially a Williamson synthesis. The coupling process produces the sodium salt of 2,4-D acid. The 2,4-D is released by acidifying with HCl. Final product is a white to tan granular solid and contains about 1% impurities including NaCl.

EQUIPMENT:

One 1,500 gallon stainless steel, agitated and jacketed coupling reactor.

One 1,200 gallon steel, agitated and jacketed coupling reactor which is generally used with the recovered DCP and TCP.

MCA, DCP and HCl are stored in tanks as mentioned in previous sections.

50% Caustic storage tank, steel - 12,000 gallons.

Weigh tank to measure DCP, glassed steel on platform scales-300 gallons.

Caustic drop tank, steel - 350 gallons.

Rotary vacuum drum filter.

Auxiliary items such as vacuum pump, etc.

Rotary drum filter feed tank, wood, with agitator - 5,000 gallons.

Slurry tank to hold cake from rotary drum filter, heated and agitated.

Filtrate tank for liquor from rotary drum filter - 7,500 gallon.

Acidification equipment including:

Small stainless steel mix tank with agitator - 75 gallons.

Cooling tank, steel - 350 gallons.

Large vacuum filter, Cypress wood, similar to a large Buchner funnel.

Carts to convey wet 2,4-D acid to drying area.

Atmospheric tray dryers. (Screen bottom trays)

Miscellaneous pumps, etc.

METHOD OF MANUFACTURE:

DCP, water, and half of the caustic pumped to coupling reactor.

Initial temperature held at less than 55°C.

MCA and the remainder of the caustic then added with cooling, pH of 10.0 to 10.5 important.

Temperature allowed to go to 75-85°C.

Mixture now contains various products including sodium chloride, sodium glycolate, sodium dichlorophenate, sodium salt of dichlorophenoxyacetic acid.

The coupling reaction takes approximately 8 hours.

Material pumped to rotary vacuum drum filter feed tank along with more water.

Slurry filtered on rotary vacuum drum filter.

Filtrate containing unreacted DCP, sodium chloride, degraded monochloroacetic acid and some dissolved sodium salt of 2,4-D acid pumped to a storage tank. The 2,4-D and DCP are recovered by pH adjustment and toluene extraction.

Cake from the rotary drum filter washed and dumped into a slurry tank. This contains the sodium salt of 2,4-D acid.

The cake slurry is pumped to a mix-tank and adjusted with HCl on a continuous basis to maintain a pH of less than 1. This releases the 2,4-D acid.

The adjusted slurry fed by gravity to a large Buchner type filter.

Filtrate goes to wash tank. pH adjusted. Secondary filtrate to sewer.

The cake is washed to remove most of the NaCl and the wet 2,4-D acid shoveled into carts.

The carts are moved to the drying areas and the 2,4-D acid dryed for use later in the esterification operation. Wet product is used when formulating amines.

Obtain about 4,000 pounds 2,4-D acid per batch.

CAPACITY:

Currently about 200,000 lbs./month.

UNIT QUANTITIES (per 100 lbs. 2,4-D)

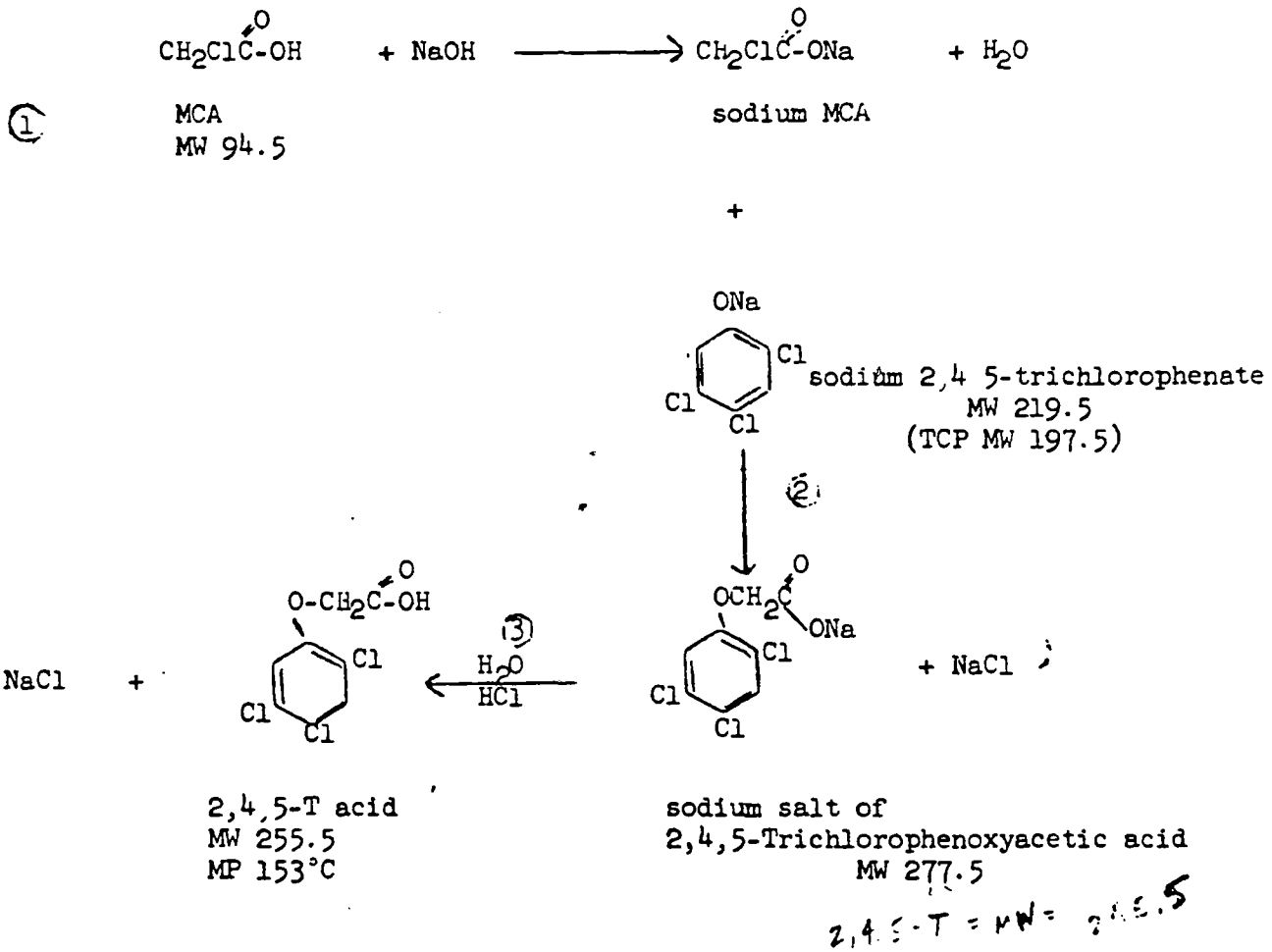
	Theory	Actual	AVG. 67
DCP	73.8	*105	74.56
MCA	42.7	64	50.0
NaOH (50%)	73	**125	56.5 (100%)
HCl as 32% (by-product from DCP & MCA operations)		165	46.7
Toluene (gal)	-	4.3	

*This should drop to 82 assuming 90% receovery of DCP.
**Excess caustic used.

2,4,5-TRICHLOROPHENOXYACETIC ACID (2,4,5-T)

CHEMISTRY:

2,4,5-Trichlorophenoxyacetic acid is prepared by coupling monochloroacetic acid with trichlorophenol (in a sodium salt solution). The reaction is summarized below:



2,4,5-T = MW = 255.5

As with the 2,4-D reaction this is essentially a Williamson synthesis for amyl-alkyl ethers. The coupling process produces the sodium salt of 2,4,5-T acid. The 2,4,5-T is released by acidifying with HCl. Final product is a tan granular solid and contains about 2% impurities such as tetrachlorobenzene and sodium chloride.

EQUIPMENT:

One 750 gallon stainless steel, agitated and jacketed coupling reactor.

MCA, HCl and caustic are stored in tanks as mentioned in 2,4-D section.

Rotary vacuum drum filter with auxiliary equipment such as vacuum pump, feed tank, filtrate tank, and cake slurry tank.

Acidification equipment including:

Cypress wood mix tank with agitator - 100 gallons.

Cypress wood cooling tank - 200 gallons.

Large vacuum filter, cypress wood - similar to a large Buchner funnel.

Carts to convey wet 2,4,5-T acid to drying area.

Same atmospheric tray dryers as used with 2,4-D.

Miscellaneous pumps, etc.

METHOD OF MANUFACTURE:

Essentially the same as 2,4-D except less caustic required due to excess amount already in the TCP slurry.

Cycle time for coupling about 8 hours.

Separation made as with 2,4-D.

Obtain about 1,100 pounds 2,4,5-T acid per batch.

CAPACITY:

Currently about 70,000 lbs./month.

UNIT QUANTITIES (per 100 lbs. 2,4,5-T)

	Theory	Actual	AVG. ?
TCP	77.3	*111	79.28
MCA	37	60	44.82
HCl (as 32%)	-	150	42.7? (20°Bé)
Toluene (gals)	-	6.0	?9.3 ±
NaOH (50%)	-	50	52.7

*This should drop to 86 assuming 90% recovery of TCP.

4

PHARMACOL. REVIEW
14:225(1962)

TOXICOLOGY OF HERBICIDES

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TABLE OF CONTENTS

I. Introduction 225
II. Inorganic herbicides 226
A. Arsenites 226
B. Sodium chlorate 227
C. Sulphuric acid 227
III. Organic herbicides 227
A. Chlorinated phenoxy-acids 227
B. Chlorinated aliphatic acids and their sodium salts 231
C. Carbamates and allyl alcohol 234
D. Substituted ureas 236
E. Triazines 238
F. Substituted phenols 240
G. Miscellaneous organic herbicides 241
1. Tributyl phosphorotrithioate (DEF) 241
2. Maleic hydrazide 243
3. Endothal sodium 243
4. Diquat (Reglone) 244
5. Aminotriazole 244
IV. Conclusions 245

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

5952

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

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 concentration. 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

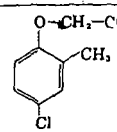
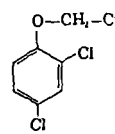
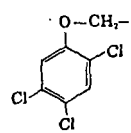
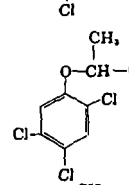
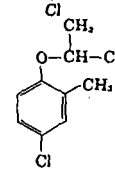
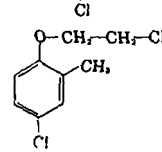
A. Chlorinated phenoxy-acids

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-phenoxypropionic 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)

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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

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	(85)

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).

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 5320 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 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

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 isopropyl 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).

toxicity to rats and rabbits, LD50 by oral administration [illegible] 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 (115).

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 1000 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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Diamond Alkali Company

INTER-OFFICE CORRESPONDENCE

5/26/69

TO: L. L. Cecil

FROM: R. J. Harvey

SUBJECT: CUTHOR COMPLAINT
(Our File No. 1312)

CONFIDENTIAL

Larry, as you can see by Bob Linderman's memo of April 26, 1969 this problem just does not involve quality of product as a factor.

This dermatitis problem is a potential hazard to anyone working with such products as the chloro-phenoxy herbicides. This is especially so of 2,4,5-T. It is not unique with Diamond 2,4,5-T but is an inherent danger with all 2,4,5-T products.

This entire problem has been investigated and is continuing to be investigated by all major producers of these chemicals. This also entails the study of penta-chlorophenol and trichlorophenol etc. Theories are abundant, as are facts. But unfortunately no one has a hard and fast answer to the problem.

Last year, in fact, I observed a similar case as Quiver describes near Philadelphia. It is fairly sure we were not obliged to do much locally; however, we did lose the customer. Some competitors will use an outcrop such as this to their advantage by claiming their products are safer.

This is not so. For example I believe Amchem has had 3-4 complaints along these lines the past year in the Northwest. Monsanto has had problems in their West Virginia plant and Dow has had this problem on and off in their plants in past years.

The logical question then is just what can be done in this connection? Here are some facts which may be of some value to you.

(1) Men under 30 are more prone to chloracne than older men.

(2) People with oily skin and coarse pores are more prone to this dermatitis problem than people with dry skin and fine pores.

(3) Cleanliness is essential. All spray applicators should wash thoroughly with soap & water following spraying.

(4) Protective clothing should be worn while spraying.

(5) If the applicator has the material blown onto him he might do well to stop, wash, and change clothes.

(6) Frequent clothing changes are desirable

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Cont.

PLAINTIFF'S EXHIBIT 2

5958

MEMO: L. L. Cecil

SUBJECT: CUTHOR COMPLAINT
(CUTHOR'S ORDER NO. 1312)

CONFIDENTIAL

May 2, 1963

(7) Some people, with all the above in mind, are so much more sensitive, anyway.

I hope that this information will be of some value to you. If the people I've copied feel any additional comments or corrections are necessary I'm sure you will be hearing from them.

Dick.

R. J. Marrese.

cc: R. A. Ondi
J. O. King
E. L. Chandler

* This problem is more likely to occur when using 2,4,5-T than when using 2,4-D. In fact, some people cannot work with 2,4,5-T.

DS00017941

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5960

6

7

THE DOW CHEMICAL COMPANY

MIDLAND, Michigan
March 29, 1965

Copy to Bill Haberl on 1-22-69 - OSCR.

V. K. Rowe
Biochemical Research Laboratory
1701 Building

cc: F. H. Riley
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K. C. Barrons
H. R. Hoyle
B. B. Holder, M.D.
S. E. Sadek

0019193

REPORT ON THE CHLORACNE PROBLEM MEETING ON 3/24/65

Present: Dr. J. Wilkenfeld and
Mr. Raymond Verhoeze, Hooker Chemical Corporation

Mr. Francis Kennedy and
Dr. Ed Chandler, Diamond Alkali Company

Mr. C. L. Dunn and
Dr. John P. Frawley, Hercules Powder Company

V. K. recapped the Dow situation in terms of the problem and the initial studies by Toxicology and Environmental Research Laboratory regarding the in-plant situation. He expanded this in general terms to the study of end products, ours and other peoples. He made reference to symmetrical tetrachloro-p-dibenzodioxin. He referred to the evidence for unknown acnegens. There were some questions from the group about the unknowns. We (Dow) were not able to answer these questions except to review the evidence for their existence in the process samples and end products.

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D.C., E.D. Mi. 4-4-73; DOW/EPA AGREEMENT 9-79

PLAINTIFF'S EXHIBIT 15

Dr. Holder reviewed the medical side of the Dow experience; he said that we now have approximately 60 to 70 cases of individuals with chloracne ranging from two severe cases to some very mild cases that were difficult to diagnose. He showed slides of the more dramatic cases. The slides were exclusively views of the faces of the individuals afflicted. He described in fair detail the appearance of the individuals mentioning the blackheads specifically. He then reviewed the clinical studies that are being made on these people with emphasis on the liver function tests. He mentioned the single liver biopsy that has been taken and studied in which the liver was normal although the man had a rather pronounced case of chloracne. Dr. Holder also mentioned the incidence of fatigue among the afflicted people as being the only other significant finding in these folks. He touched briefly on treatment indicating that various topical treatments were not particularly effective. He described the cycling of this disorder in individuals who had been completely removed from exposure. He mentioned that some fellows are approaching the end of their trouble two or two and one-half years after onset of the skin disorder. He also described "acute chloracne" which is an acute inflammatory condition that appears considerably sooner than the normal chloracne in individuals and appears after pronounced single exposure. The acute chloracne shows up within a few days of exposure. Dr. Holder mentioned five to eight days specifically. There was considerable discussion by the group on the skin disorder itself. The Hooker representatives related experience of skin condition thirty years after exposure. Their cases were more similar to the Dowicide bumps which Dow has experienced in that there were large boils or large bumps rather than the multitude of small blackheads and eruptions which Dow is seeing in the current cases.

Dr. Sadek showed slides of ears and livers of rabbits that had been exposed to the symmetrical tetrachloro-p-dibenzodioxin. He discussed the pathology in detail which I will not attempt to summarize.

V. K. mentioned the studies in which the rabbit ears have been treated with TCBD in benzene or corn oil and then washed with soap and water at various time intervals later. If exposure occurs for very long, washing does little good. He also briefly mentioned the oral studies but without detail. Silverstein described the plant study on washing of contamination from tools and surfaces. This study indicated that benzene, acetone and Chlorothene NU were effective in removing the contaminant from tools and also that detergent and water with scrubbing action could clean up tools and equipment. Some discussion ensued on the use

of detergent and water and the point was made again that strong scrubbing action was necessary for this approach to be successful.

Harold Gill then discussed the analysis for tetrachloro-p-dibenzodioxin by vapor phase chromatography. He listed the limit of sensitivity on various process materials. He mentioned the oil which he defined as a non-saponifiable mixture of chloro anisoles, tetrachlorobenzene and trichlorobenzene; the limit of sensitivity for TCBD in this material is 10 ppm. The limit is 1 ppm for 2,4,5-trichlorophenol, and for 2,4,5-T Acid, either acetic or propionic. Gill then defined 1 ppm as a very discernible peak. He mentioned that he might estimate 0.5 ppm in some instances but to be conservative the analyst reports <1 ppm if the peak does not measure up to the quite identifiable level of 1 ppm. The analytical problem has not yet been solved for the T-Acid esters. The general procedure used for the T-Acids is to extract the sample (arbitrarily about 20 grams) with chloroform (about 40 milliliters), filter the chloroform to remove solids and wash with an equal volume of N/10 caustic to remove any acidic materials. The chloroform extract then is concentrated by evaporation to one-tenth the original volume; thus, the concentration of the dioxin in the chloroform will be ten times higher than in the original sample. When the analysis is conducted on trichlorophenol, the material is dissolved in N/1 caustic to the extent of 10%, and this solution is then extracted with the chloroform and handled as indicated above.

A question was asked about the utilization of detectors other than the flame ionization which is specified in the Analytical Laboratory write-up for this analysis. Gill has not tried the micro coulometric detector because he is not set up to do so, but he has experimented with electron capture. He stated that theoretically this unit should not provide any greater increase in sensitivity. In actuality he found a slight increase in sensitivity but there are usually too many chlorinated species present which may saturate the electron capture cell whose recovery is too slow to be of practical use. He summarized by saying that the slight increase in sensitivity is not worth the effort to switch from flame ionization to electron capture. A question was asked about how the extraction is performed. Gill stated that it is performed in a wide mouth bottle on a shaker for one hour. (It was not mentioned, but it is the case that this is done at room temperature.) He mentioned that spiked samples have been

run this way and the recovery ranged from 90 to 100 per cent. The ratio of solvent to the material being extracted on this step is not critical according to Gill. Their standard procedure is 20 grams of sample and 40 milliliters of chloroform. On trichlorophenol samples specifically, 20 grams of phenol is converted to phenate — about 10 per cent concentration in water. The phenate solution is extracted with 20 milliliters of chloroform in a single extraction. The chloroform is then concentrated so that the concentration of the dioxin will be ten times that in the original sample.

The question of volatility of dioxin came up and Harold Gill stated that he found he can distill o-dichlorobenzene away from tetrachlorobenzodioxin. He said that in his opinion the secret was to avoid distilling to dryness.

A member of the group asked if samples of standard TCBD were available. The answer was yes and 100 mg samples were provided to one of the representatives from each company. (A sample had previously been given to Dr. Kelly of Monsanto.) A question of laboratory safety in the analytical work came up and the basic precaution of wearing vinyl gloves was mentioned. Information relative to the gloves we used was provided to the group.

Disposal of contaminated laboratory materials and plant materials was discussed. We mentioned that Dow burns some small amounts of waste. Harold Gill stated that his laboratory study of combustion showed that 99.96 per cent of the dioxin sample was burned at 800°C. We described why we felt that our practice of burning small amounts of dioxin was a safe one.

V. K. then outlined the project in which plant samples and products (not mentioned by name) were spiked with known amounts of the TCBD. The spiked samples were split for the purpose of checking our analytical procedures for recovery and correlating these results with the bio-assay method.

The question of specification, quality control specification that is, was raised and we were asked if we could give levels of dioxin contamination which were permissible limits. V. K. mentioned that at present we are using zero with a confidence of 1 ppm in process samples. There was some discussion on the problem of customers using finished products under far less desirable conditions of health control than we can provide our workmen in our own plant. There seemed to be agreement among the group that we could not afford to sell contaminated products.

Jack Peterson then discussed the data from animal experiments using pure symmetrical tetrachlorobenzodioxin. Doses ranging from 2 parts per billion to 1000 parts per million of tetrachlorobenzodioxin in benzene had been administered to the rabbit ear. Dosage in most cases was 0.1 ml per day. Both single and multiple exposures have been studied and multiple exposures administered on a five days per week basis. The significant factors in the study are dose, the number of applications and the days on exposure of the animals. The response which is reported in the gross observation of the condition of the rabbit's ear by the toxicologists. This does not include pathological findings -- there is not enough data in this area to discuss. The level of response ranges from none through very slight, slight, slight to moderate, moderate, moderate to severe, severe, and extremely severe. Jack indicated to the group that there is not a sharp definition between these categories of response and indicated also that there is some difficulty in graphing this type of response. He described the response from single applications to the rabbit ear first: at 100 parts per million there was a severe response in eight days; at 40 parts per million there was a slight response in eleven days; at 20, 10, 7 and 4 parts per million there was no response. These tests were run on single rabbits and without washing the material off. Jack then discussed the multiple application data which he took from his major graph of this data. The important points that he made from this data were first that at the limit of VPC sensitivity a severe response may be produced. In other words, even if the VPC does not detect TCBD, an animal response may still occur. His second important point was that the induction period for response averaged about ten days on the animals in the studies.

There was a brief discussion then about the air samples that were taken in the plant. Silverstein mentioned that some air samples have shown activity on the animals. The degree of response is slight and the number of samples that show activity is small out of the total number taken and the amount of air that must be sampled is very much larger than the amount a man normally breathes in an eight hour day.

The meeting was adjourned. The group then proceeded to the Toxicology Laboratory to view some of the test animals. They were shown responses of varying intensity and these were described. This demonstration appeared to have considerable impact.

L. G. Silverstein
Biochemical Research Laboratory
1701 Building

LGS:sjl
5966

Postscript

All participants seemed to appreciate well the problem and all indicated that they would return home and attempt to convince their management to institute safety specifications (really quality control) for their various products in this area. All agreed that the industry should meet its own responsibility. All were very appreciative of Dow's effort to steer them away from a danger area. Time will tell whether we accomplished our mission, but as of now I feel satisfied with our effort and the reception it received.

VKR

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V.K. Rowe 6/24/65

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DEFENDANT'S EXHIBIT 1623

DEPOSITION EXHIBIT 25 Silbergeld

MIDLAND
June 24, 1965

DOW CONFIDENTIAL

MM0697799

DOW 7470996

Ross Mulholland
Manager
Bioproducts
Dow Chemical of Canada
Sarnia, Canada

2,4,5-TRICHLOROPHENOL, THE "T" ACIDS, AND ASSOCIATED ACHESENS

I have not been neglecting your request for information to use in discussing the subject problem with Saugatuck and the Co-Op. I have been stymied, however, because the analytical methods have been changed and are in the process of being cleared and reproduced. I expect them any day, but rather than wait longer, I thought I should advise you of the situation. I will send you copies of these methods as soon as they become available.

In regard to the overall problem, we are attempting to do everything possible to avoid the possible occurrence of chloracne in any applications involving the handling or use of trichlorophenol, trichlorophenoxyacetic acid and its derivatives. As you well know, we had a serious situation in our operating plants because of contamination of 2,4,5-trichlorophenol with impurities, the most active of which is 2,3,7,8-tetrachlorodibenzodioxin. This material is exceptionally toxic; it has a tremendous potential for producing chloracne and systemic injury. If it is present in the trichlorophenol, it will be carried through into the T acid and into the esters and hence into formulations which are to be sold to the public. One of the things which we want to avoid is the occurrence of any acne in consumers. I am particularly concerned here with persons who are using the material on a daily, repeated basis such as custom operators may use it. If this should occur, the whole 2,4,5-T industry will be hard hit and I would expect restrictive legislation, either barring the material or putting very rigid controls upon it. This is the main reason why we are so concerned that we clean up our own house from within, rather than having someone from without do it for us. In this way, we can approach the problem in an orderly manner. If the producers and handlers of this material will cooperate, there is no reason why we cannot get this problem under strict control and thereby hopefully avoid restrictive legislation; in other words, let us practice good citizenship. At the present time, we are of the opinion that material containing no tetrachlorodibenzodioxin with a certainty of 1 ppm does not present an appreciable hazard to consumers; likewise, we do not believe that such material constitutes a significant hazard to persons working in

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EXHIBIT Rowe-14 FD 3-31-84 J.F.

p'ants handling such phenol, T acid, or T acid esters.

I might add that we are continuing our researches on this particular problem from the standpoint of studying the other impurities which may have the capacity to produce this type of reaction. Also, we are attempting to quantitate the effects of the known acnegens when added to base materials. This work is progressing well, but it will be several months before we have a completed story.

I would urge again that if your big customers such as Co-Op and Naugatuck have particular questions about this problem that you invite them to come to Midland where we will be glad to discuss the matter in detail with them and show them what we have learned. We are not in any way attempting to hide our problem under a heap of sand, but we certainly do not want to have any situations arise which will cause the regulatory agencies to become restrictive. Our primary objective is to avoid this.

I trust that you will be very judicious in your use of this information. It could be quite embarrassing if it were misinterpreted or misused.

V. K. Rowe
Biochemical Research Laboratory
1701 Building
MX 6-2376

VKR/jd

cc: L. Silverstein
C. Otis
Grady Holdeman
F. Arntzen
G. Goergen
H. Hoyle
W. Falsey
V. K. Rowe (2)
T17.4-12-20
Correspondence

P.S. Under no circumstances may this letter be reproduced, shown, or sent to anyone outside of Dow.

VKR

DOW 747097

DOW CHEMICAL U.S.A.
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0000005 KD

B. JOHNSON

MAR 24 '83

Ex. William Thompson-3

February 15, 1967

DOW 7518711

Mr. M. S. Buckley
Technical Director
Thompson Chemical Company
3023 Locust Street
St. Louis, Missouri 63101

Dear Mr. Buckley:

Pursuant to our discussion a week or so ago about your chloracne problem, I have had three published articles reproduced and am enclosing them herewith. Two of them are translations which we have made. The translations may not be perfect, but the important points are there. One is entitled "Clinical Observations about the Origin of Chloracne," and the other is entitled "Industrial Poisoning in the Manufacture of Chlorophenol Compounds." In this latter article, you see reference to 2,4-dichlorophenol as well as to 2,4,5-trichlorophenol. I have a feeling that this is a mistake, and that this reaction is not typical of 2,4-D manufacture. References to the original articles are given so that you can go back to the original if you see fit. However, I thought that inasmuch as the translations were available, it might save you time and money in getting acquainted with the history of this ugly disease. The title of the third article included is "Industrially Acquired Porphyria."

I trust that this information will be of value to you.

Sincerely yours,

5972

V. K. Rowe
Biochemical Research Laboratory
1803 Building

Enclosures

PEW

bcc: W. J. McCoy
W. R. Dixon
B. B. Holder, MD
L. Silverstein
V. K. Rowe (2)
Correspondence
T17.4-23-20

B. JOHNSON

MAR 24 '83

ex William Thompson 4

DOW7515T3

Transl. MID. no.5728

Hautarzt
Vol.10 no.3 p.126-129 (1959)

Clinical observations about the origin of chloracne.

Author: Braun, W.

Translator: Trans. Chem. Inc.
Date: - May 27, 1966

Requested by: Murray, Biochem., 1701 Bldg.
Original language: German
Translation no.: 66-3-46

Copy sent to
M.S. Buckley at Thompson
on 2-15-67
- OK

17 - Dunn

0000345

HERCULES POWDER COMPANY
INCORPORATED

cc: J. M. [illegible]gan - Syn.
M. A. Taves - R. C.
J. J. Ford - R. C.
A. Z. Conner - R. C.
A. D. Sidwell - Jacksonville

Wilmington, Delaware
November 18, 1965

TO: C. L. Dunn - Synthetics Department

FROM: E. E. Christofano - Medical Department

The following comments will summarize the investigations we contemplate on chloracne possibly associated with 2,4,5-T esters.

1. Badische uses a bioassay technique to check each batch of TCP purchased. Their test consists of feeding each of three rabbits with 1 gm./kg. of TCP, with a determination of bromsulphalein retention after 5 days. More than 5% of BSP retention is cause for rejection of the batch. Sensitivity is claimed at 3 ppm.

2. Dow bioassays by a rabbit ear painting technique. The internal surface of one rabbit ear is painted daily with 0.1 ml. of a chloroform solution of test material (10 or 50%) of an extract of TCP in chloroform. Unless severe irritation is noted 10 doses are applied. The ears are observed for two additional weeks for the appearance of folliculitis and cyst formation. They have noted fatalities from a single application and liver damage has accompanied the chloracne reactions. Sensitivity is claimed at about 1 ppm.

While the Dow testing may be more sensitive, the Badische method is faster and less expensive. In order to evaluate both methods we recommend that Hercules supply material to Badische for their testing and simultaneously begin gathering confirming data in a commercial laboratory. Because of a lack of information on the toxicity of in-process materials, these latter studies should begin only after oral toxicity has been determined. For this first stage the acute oral LD_{50} studies would cost approximately $300. Subsequent acnegen studies on these materials are expected to cost about $6,000, however, an accurate estimate is not possible until Stage 1 is complete. Preparation of the samples and subsequent analysis is estimated to cost $500.

DEFENDANT'S EXHIBIT 1638

HERCULES

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DEPOSITION EXHIBIT Dunn 11 6-27-90 NF

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Dr. Oettel of Badische has agreed to bioassay several spiked samples to permit comparison of results with the gas chromatographic determination of dioxin and with the Dow ear painting procedure. To permit this study we request that 25 g. of each of the following spiked samples be prepared:

a. Standard 2,4,5-T acid spiked with 0, 2, 5, 10, 20 ppm dioxin.

b. Plant grade 2,4,5-T acid with a known dioxin content.

c. Plant grade sodium trichlorophenate spiked with 0, 1, 3, 5, 10 ppm dioxin (based on TCP equivalent).

d. Dioxin standard approximately 2 mg.

e. Plant grade propyl ester of 2,4,5-T acid as 6 lbs/gal. solution in xylene spiked with 0, 2, 5, 10, 20 ppm dioxin.

Approximately 10 gms. of each sample should be prepared for shipment to Dr. Oettel and the remainder retained for future confirming tests conducted by Bio-Test. Chemical analysis of the spiked samples would be required after the biological testing demonstrates physiological activity.

Acute oral toxicity data on sodium trichlophenate, 2,4,5-T acid and finished propyl ester formulation should be begun promptly. Approximately 50 gms. of each material should be sent to Dr. Joseph C. Calandra, Industrial Bio-Test Laboratories, 1810 Frontage Road, Northbrook, Illinois. Dr. Calandra has a supply of dioxin.

After these preliminary tests are conducted we will establish a more comprehensive program for the evaluation of hazard during manufacture and in the product as it would be used by the customer. Without these preliminary data a test program would be unnecessarily expensive.

E Christofano

EEC:vvh

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RESEARCH DEPARTMENT
HERCULES INCORPORATED
RESEARCH CENTER

cc: Mr. J. H. Eagan - Sym.
Mr. C. L. [illegible]nn - Sym.
Dr. H. A. Taves
Mr. A. Z. Conner

0000347
K

Wilmington, Delaware
June 13, 1966

TO: MR. E. E. CHRISTOFANO, MEDICAL DEPARTMENT

FROM: J. J. FORD, ANALYTICAL DIVISION

DIOXIN FORTIFIED SAMPLES FOR BIOASSAY TESTS

In accordance with your letter of November 18, 1965, the samples listed below are being sent to you for transmittal to Dr. Oettel of Badische, for use in the bioassay tests.

Approximately 10 grams of each sample (except for the pure dioxin sample) is included. The samples are packed in four shipping containers, and the contents of each are listed on the outside of the container. The individual samples are also clearly identified with an appropriate label. I have indicated which samples have dioxin added to them, but have not shown the fortification level on the label. If you wish to provide Dr. Oettel with this information, it is included in the attached list. It would be useful to point out to Dr. Oettel that the samples from Group 3 and Group 6 are more easily sampled if heated as indicated.

J. J. Ford

JJF/cbb
Attachment

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DEPOSITION EXHIBIT
Dunn 12
6-27-90 PE

DEFENDANT'S EXHIBIT
1639

SAMPLES PREPARED FOR BIOASSAY TESTS

(1) Standard 2,4,5 T Acid With Added Dioxin

Sample Designation	ppm. Dioxin Added
X14867-41-1	0
X14867-41-2	2
X14867-41-3	5
X14867-41-4	10
X14867-41-5	20

(2) Plant Grade 2,4,5 T acid with Known Dioxin

Sample Designation	ppm. Dioxin
X15280-56-8	3
X15280-56-9	1

(3) Plant Grade Sodium Trichlorophenate with Added Dioxin
Fortification is based on a 35% NaTCP Content.

Sample Designation	ppm. Dioxin
X15280-55-1	0
X15280-55-2	1.2
X15280-55-3	2.4
X15280-55-4	5.7
X15280-55-5	13.0

Heating the above samples to 65°C. aids in sampling.

(4) Dioxin Sample - 2,3,7,8 tetrachlorodibenzo-p-dioxin - Designated X15280-56-10.

(5) 2 Ethyl hexyl ester of 2,4,5 T with added dioxin (Brush-Rhap LV-4-O) Fortification is based on 44.1% 2,4,5 T acid.

Sample Designation	ppm. Dioxin
X15280-56-1	0
X15280-56-2	2
X15280-56-3	5
X15280-56-4	10
X15280-56-5	20

(6) Non-saponifiable fraction from wash solvent still. Designated X15280-56-7. This sample contains unreacted tetrachlorobenzene and relatives, anisol of trichlorophenol and related phenols, toluene and a small amount of NaTCP. Sidwell believes this is where the dioxin would end up in our process. We are storing this material at the rate of 2-3 drums per day. To melt, heat this sample to 50°C.

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S. JOHNSON

MAR 24 '83

Ex. William Thompson 2

MIDLAND
February 15, 1967

DOW 751872

William E. Dixon
Bioproducts Department
BPC

cc: W. L. Corbin, BPC
D. E. Fletcher, BPC
J. H. Cowell, BPC
W. J. McCoy, BPC
B. B. Holder, M.D., 607

THOMPSON CHEMICAL COMPANY, ST. LOUIS, MISSOURI

On February 2, 1967, W. J. McCoy informed me that a Mr. M. S. Buckley of Thompson Chemical had called Dow indicating that they had had an accident associated with the manufacture of trichlorophenol, and that they had some men with what they thought was chloracne. They wondered if we could advise them in regard to medical practice. I contacted Dr. Holder, who then called Mr. Buckley and discussed with him medical aspects of the problem. It is my understanding from Dr. Holder that Mr. Buckley then started talking about the chemistry of this material, and Dr. Holder suggested that he contact me in regard to anything along this line.

On or about February 7 Mr. Buckley called me to see what we knew about chloracne caused by contact with materials associated with the manufacture of trichlorophenol from tetrachlorobenzene. I told him that under certain circumstances, which I did not describe, the caustic insoluble oils could contain considerable amounts of a very highly toxic substance which we had identified as 2, 3, 7, 8-tetrachlorodibenzodioxin. I indicated to him that this material was not only extremely toxic systemically, but it was also an extremely potent chloracnogen. He indicated that they had been distilling some oils or tars containing anisoles when the operation got out of control, and the material either spilled or splashed onto a hot autoclave. He thought that the fumes and vapors of the anisoles had caused their difficulty. I told him I did not think the acne was caused by the anisoles, but rather by other materials present in the anisoles.

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DOW751871

Apparently Mr. Buckley had asked Dr. Holder how to clean up the equipment, etc., and Dr. Holder indicated to him that he must take extreme precautions, including the use of rubber suits and gloves and respirators. He seemed surprised that we would recommend such severe measures, but I reiterated that such precautions were what we believed necessary. He then wanted to know if I thought they could clean up the contaminated equipment with steam. I felt at this point an obligation to tell him that steam was the worst thing he could use, simply because it would volatilize the material, causing it to recrystallize and deposit elsewhere. This was not the way to get rid of it. He suggested scrubbing down the equipment with detergent or solvents. I indicated that this could be done if he made sure that all necessary precautions were taken to prevent contact with the people. I suggested that he assay or take wipe samples of the equipment to determine the degree of contamination. I suggested that this should be done before and after the clean-up. He asked how such assays could be made. I indicated to him that we had developed a method of analysis and also used biological assay employing a rabbit ear. He did not ask me for details of these procedures, so I did not give them to him.

He then asked where he could find information about chloracne, and I volunteered to send him copies of pertinent articles from the published literature which I had, and this has been done.

My conversations with Mr. Buckley were within the framework of our discussion of the problem. The information on the physiological activity was perhaps even less than was given to the other producers of trichlorophenol over a year ago. This was because it was quickly apparent that Mr. Buckley had little understanding of the toxicological aspects of his problem. Had he asked for methods, etc., I would have agreed to send them to him. However, since he did not, I saw no reason to volunteer.

I don't know whether this information is of any value to you, but it will, at least, keep you informed. If you have any questions or suggestions for further action, please do not hesitate to contact me.

5981

V. K. Rowe
Biochemical Research Laboratory
1803 Building

psw

bcc: V. K. Rowe (2)
T17.4-23-20
Chloracne file T 36
Correspondence

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HERCULES INCORPORATED

Confidential
7/5/67 JB
DC
AEG
HEW
Al - please discuss with me and then I'll see HEW

cc: Dr. J. P. Frawley - Medical Dept.

Wilmington, Delaware
June 30, 1967

TO: Mr. H. E. Wilder - Jacksonville

FROM: J. M. Eagan

Dr. Frawley sent to me the attached copy of a report dealing with five cases of cloracne in Colombia which developed from the use of phenoxy herbicides. The inference in these reports is that some producers allowed contaminated 2,4,5-T to enter sales channels in 1963 and 1964. No prior cases of cloracne outside of manufacturing operations have come to our attention but this report should remind all of us that we must be certain to continue to be on the alert for abnormal operating conditions which might contribute to the presence of the chemical which is reputed to be responsible for this type of cloracne.

JME/abk
Attachment

J M Eagan

5983

104-25

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN 48640

September 26, 1967

Colonel C. G. Shead (SAO)
Directorate of Air Force Aerospace Fuels
Kelly Air Force Base, Texas 78241

Dear Colonel Shead:

We have been asked to bid on the design, conversion and operation of an 8,000,000 gallon per year "Orange" plant at the Government owned chemical plant at Weldon Spring, Missouri.

This is to inform you that we have advised W. G. Kratz, Colonel, Corps of Engineers in Kansas City, Missouri, that we do not intend to bid on their solicitation No. DACA41-68-R-0001, dated September 7, 1967.

"The basic reason for our decision is due to critical factors outlined below:

"The time allotted for submission of bids, September 8, 1967 to October 12, 1967, is not adequate for preparation of bids on a project of this magnitude.

"The presentation of alternative design at locations other than Weldon Spring were precluded because of the time allowed for preparation of a responsive bid in answer to this solicitation.

"We would like to sum up some other reasons for not presenting a bid at this time, as follows:

1. The Weldon Spring site, located as it is between the University of Missouri Experimental Farm and the August Busch Reserve, presents too much of a hazard to the plant life in these areas, in case of an accidental release of products from the Orange Plant.

2. The requirement of 15 months start up and full operation in 18 months on the Weldon Spring site appears almost impossible to meet without excessive overtime.

3. The labor situation in the St. Louis area would present too many problems.

4. It would be almost impossible to solve the pollution problems in the area.

BATCH 513 FSA JJS 0063

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BC BATCH 513 FSA (JUS) 0063

Colonel C. G. Shead — September 26, 1967

5. Disposal of waste could not be solved in time to arrive at a positive solution before the time allotted for completion of the plant.

6. The Chlor-Acne problem associated with the manufacture of Trichlorphenol could not be solved without first receiving permission from a European Chemical Manufacturer.

   The Chlor-Acne problem is one of Human Health which Dow has had to combat in the design of its own plants at its Midland operations.

7. Several phases of the manufacturing process for 2,4-D and 2,4,5-T involve Dow know-how that we do not wish to lose to our competitors.

8. The people that are now engaged, at the factory level, in the production of 2,000,000 gallons/year of Orange for the Government are the same people that would have to be assigned to the White Spring project.

"The Dow Chemical Company continues to have a high degree of interest in the project and requests that we be kept on your bidders list for any following solicitation in connection with the supply of raw or intermediate materials.

"When the TCB RFP is released we hope to be on the bidders list, and feel that we will be in a better position to bid on this project.

"If you have any questions concerning Dow's decision with regard to Inc. DACA31-68-R-0001, please contact us."

Sincerely yours,

A. P. Brutel

A. P. Brutel, Vice President
Director of Government Affairs

October 16, 1968

Mr. V. K. Rowe
Biochemical Research Laboratory
The Dow Chemical Company
1701 Building
Midland, Michigan

Dear Mr. Rowe:

As you may recall, Hercules has been attempting to develop a bioassay technique to simplify detection of dioxin in herbicide formulations. Based principally upon work initiated by Dr. Oettel at BASF, the tests measured liver function changes which result from feeding rabbits with herbicide. We feel such a technique offers several advantages over rabbit ear painting tests.

Unfortunately, we have been unsuccessful. But to state it so bluntly is really unfair. First, we attempted to determine the sensitivity of liver change by feeding test materials with known additions of dioxin. The toxicity of test materials prevented use of liver function change as a bioassay for dioxin even at 10 ppm. We then dosed animals with 0.01 & 0.1 mg/kg of dioxin suspended in corn oil. Carbon tetrachloride was administered as a positive control at 100, 250 and 500 mg/kg. Clinical determinations including SGOT, SGPT and BSP retention were conducted prior to administration and every two to three days thereafter for at least 14 days.

Significant elevations in one or more of the clinical determinations were found in some but not all dioxin animals. The changes were not well correlated with dose nor were they consistent with regard to onset or duration. Animals on the positive control test responded as would be expected with significant blood chemistry changes only two days after dosing with 100 mg/kg.

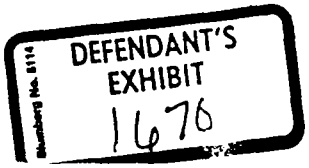
Although positive results were obtained with dioxin, the usefulness of this technique as a bioassay for the presence of dioxin is questionable.

At the Toxicology Roundtable I mentioned these results to Ken Olsen who suggested that you might comment upon them. But the other reason for writing is to solicit your opinion on the usefulness of presenting our data at the Society of Toxicology meeting next spring. Certainly such a presentation would raise questions as to the reason for conducting an unsuccessful study.

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On the other hand, someone might be spared useless effort if such negative information were offered to the scientific community.

I'd like to have your comments before we proceed.

Sincerely,

Emil E. Christofano
Industrial Hygienist

EEC:rbm

"Influence of Dioxin on Liver Function in the Rabbit,"
by M. L. Keplinger, C. P. Schoenig, O. E. Fancher,
E. E. Christofano, and J. P. Frawley.
Industrial Bio-Test Laboratories, Inc., Northbrook,
Illinois 60062 and Hercules Incorporated, Wilmington,
Delaware 19899

It was suggested that the presence of dioxin (2, 3, 7, 8 tetrachlorodibenzo-p-dioxin) as a contaminant at very low concentrations in industrial chemicals can be detected by changes in liver function following oral administration to rabbits. It was thought that this might be a sensitive bioassay for the presence of dioxin. Therefore dioxin was added to industrial chemicals at concentrations of 0, 0.3, 1.0, 3.0 and 10.0 ppm and the contaminated materials were administered orally, in doses of 80 or 100 mg/kg, to rabbits.

Determinations of SGOT, SGPT and BSP retention were made before administration and at 5 and 15 days after administration. No effects indicating clinical evidence of liver damage were found.

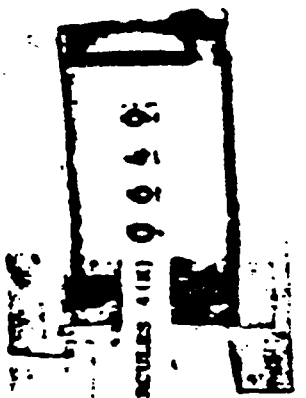
In a second experiment dioxin itself was administered at doses of 0.01 or 0.1 mg/kg (10 and 100 times the highest dose above). Carbon tetrachloride was administered at doses of 100, 250 and 500 mg/kg as a positive control. The SGOT, SGPT and BSP retention were measured every 2 or 3 days for 18 days. All three doses of carbon tetrachloride caused significant increases in SGOT, SGPT and BSP retention. Increases in these three were found in some, but not all animals receiving dioxin.

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The changes were neither well correlated with dose nor consistent in onset and duration, even in animals showing apparently significant effects.

Although some positive results were obtained with dioxin itself, the usefulness of this technique as a bioassay for the presence of dioxin is questionable.



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