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Review Section

ESTIMATION OF TOXIC HAZARD—A DECISION TREE APPROACH

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Summary—Safety evaluation is caught in a frustrating circle. It is neither possible nor sensible to try to obtain the information needed to assess every imaginable toxic risk associated with every substance, and pursuit of greater safety therefore demands the setting of priorities as well as sensible limits for investigation. To do this with confidence requires possessing the very information that is lacking and that can be won only slowly on a few substances at a time, with significant uncertainty and at considerable cost. This requires priorities, and completes the circle of frustration. Individual toxicologists deal with this problem by using 'experience', a personal synthesis of accumulated knowledge of structure-activity relationships, metabolic mechanisms, chemical reactivity, human exposure and other relevant information. Such expert judgement is often very effective in distinguishing potential risks worth pursuing from problems on which effort would be wasted but, because it is usually so inexplicit and subjective, it is seldom able to invoke the public confidence most decisions now require.

This paper proposes a procedure for making a significant part of this process rational, public and explicit. It uses much currently available toxicological data to validate the procedure, which consists of a 'decision tree' of 33 questions, each answered 'yes' or 'no'. Each answer leads to another question or to final classification into one of three classes (I, II and III) reflecting a presumption of low, moderate or serious toxicity. The tree is organized into branches dealing with major chemical classifications and is intended for use with all ingested, structurally defined organic and metallo-organic substances. Answering the questions requires chemical or biochemical training, and relies primarily on features of chemical structure. Occurrence in body tissues and fluids, and natural occurrence in food are also involved. The logic of the tree rests heavily on known data on metabolism and toxicity. The classification according to presumptive toxicity can be combined with knowledge of human intake to provide for each substance a 'protection index', which can be used to establish priorities and to define tentatively the extent of appropriate testing. The procedure has been applied to a large number of pesticides, drugs, food additives and industrial and environmental chemicals of known biological properties. So far it has not resulted in any underestimation of toxicity, and it appears to provide a practical means for discriminating effectively among different levels of probable hazard.

Background

Never since toxicology first emerged as a distinct field of applied science have the resources within the field been adequate to meet the increasing demands made on them. In part, this has been due simply to the advance of all science (Price & Desolla, 1969). W. G. Galetto (personal communication 1977) has pointed out that a count of the number of both chemical abstracts and biological abstracts between 1906 and 1976 shows an exponential growth with a doubling period of about 12 years. *Biological Abstracts* published 1,498,000 items in 1965 and about 3,000,000 in 1977. Once well-established, conclusions seldom remain undisturbed for long.

Toxicology, however, bears a double burden. We demand ever greater safety in an environment that many people fear—not necessarily correctly—is increasingly hazardous. At the same time, the notably rapid advance of analytical chemistry has continued to alert us to the presence of previously unsuspected toxicants and to a host of substances we have not yet begun to evaluate. It is clear even to a casual observer that the sensitivity and precision of analytical chemistry are far ahead of toxicology. Even less happily, toxicologists find it far easier to produce an adverse effect in laboratory animals than to interpret with assurance the meaning of such an effect for human safety.

Our response to this situation has customarily been simply to demand more testing on virtually every substance that has recently been evaluated. No one denies that we would be better off with more data. Our universal problem has been that the demand for data has grown faster than the supply. The recent passage of the Toxic Substances Control Act merely carries this trend further. At least several million dollars, in each case, have been spent on toxicological work on cyclamates, on saccharin, on Red No. 2 and on monosodium glutamate. Yet it is clear, at least in the minds of some, that from a regulatory standpoint the safety of these long-used ingredients is far from well established. Obviously, we cannot devote to every substance of present or potential interest even a fraction of this kind of effort.

This suggests some rational establishment of priorities, as a guide to the sequence, scope and intensity of investigative effort. This has several times been urged, but usually in the general terms of looking first at those substances whose structure or known biological effects raise questions, and then at the substances used in largest quantity (Joint FAO/WHO Expert Committee on Food Additives, 1976; Panel on Chemicals and Health, 1973; Select Committee on Flavor Evaluation Criteria, 1976). This is rational as far as it goes, but it is an extremely coarse screen, which fails to differentiate at all among less serious sources of potential hazard. By missing this opportunity, it leads either to the disregard of hazards of low or intermediate degree or to the unnecessary expenditure of effort on the trivial as well as the more significant.

We require therefore a preliminary assessment of probable risk—not as a substitute for data but as a guide to the priority and scope of the effort required to acquire more information. This paper attempts to suggest how information on intake currently or potentially available can be combined with a preliminary assessment of probable toxicity to provide such a guide.

We are aware of a recent publication proposing a system for selecting and defining priorities for chemicals that may present environmental hazards (Arthur D. Little, Inc., 1977). While that publication differs markedly from our paper in its scope, objectives and content, it approaches many of the same considerations with parallel concepts and language.

Toxicity has been defined as "the capacity of a substance to produce injury" and hazard as "the probability that injury will result from the use of a substance in a proposed quantity and manner" (Food Protection Committee, 1970). The primary consideration is quantity—for ingested substances, intake. Safety (the inverse of hazard) "is the practical certainty that injury will not result from the substance when used in the quantity and in the manner proposed for its use" (Food Protection Committee, 1970).

Assessment of risk, therefore, has two principal components: exposure and toxicity. There are other important considerations, including variation in individual susceptibility and the possibilities of synergistic or antagonistic effects, but in a preliminary assessment of the safety of ingested materials, intake and toxicity are paramount.

Estimates of intake of the chemical substances found naturally or introduced intentionally into food are available or potentially available largely because of the work of several agencies and organizations combined in the reports of the Subcommittee on Review of the GRAS List—Phase II (1972) of the National Academy of Sciences. Estimates of indirect-additive intakes are more difficult. The market-basket survey of the Food and Drug Administration provides actual data on pesticide residues (Food and Drug Administration Bureau of Foods, 1977). Packaging component intakes can sometimes be estimated from extraction studies that simulate 'worst cases'. Intakes from over-the-counter drugs are less readily available but those from prescription drugs are more closely controlled, although not necessarily known.

Toxicologists and pharmacologists customarily make preliminary judgements of probable biological effects, most commonly in deciding what substances to test and what effects to anticipate. Such judgements are based on knowledge or assumptions concerning structure–activity relationships and metabolic fate, as well as on presumptions that may be drawn from the occurrence of a substance in food or in body tissues and fluids. While recognizing that generalizations are difficult, and at times incorrect, we cannot avoid making them if we are to choose sensibly what to work on first and how far to go. What has been lacking is a systematic coding of these distinctions, or at least of those so commonly used that they might reasonably be expected to enjoy wide applicability and support.

This paper is a proposal in the direction of such a coding. By the use of a 'decision tree', it seeks unambiguously to classify every structurally defined organic or metallo-organic chemical by criteria that are based largely on structure or on widely known facts of biochemistry and physiological chemistry. It excludes polymers because they are not structurally defined in terms of chain length, molecular weight and cross-linking. It is concerned only with oral toxicity. It results in the placing of every such substance into one of three classes representing an estimate of toxic threat.

The tree is organized into branches, each of which deals with a broad class of compounds. Some questions are 'sorting' questions, which determine the branch to be followed. All questions lead either to another question or to a final classification. A number of terminal questions deal with aspects common to several classes of compounds and are therefore found at several branch ends. Readers may find the summary flow diagram (Fig. 1), suggested by Dr. B. L. Oser, helpful in viewing the organization and use of the tree. When one combines such an estimate of toxic threat with an estimate, or observation, of exposure, one may obtain the kind of preliminary assessment of probable risk that we are seeking. In the discussion section, we pursue such an assessment.

Class I substances are those with structures and related data suggesting a low order of oral toxicity. If combined with low human exposure, they should enjoy an extremely low priority for investigation. The criteria for adequate evidence of safety would also be minimal. Greater exposures would require proportionately higher priority for more exhaustive study.

Class III substances are those that permit no strong initial presumptions of safety, or that may even suggest significant toxicity. They thus deserve the highest priority for investigation. Particularly when per capita intake is high or a significant subsection of the population has a high intake, the implied hazard would then require the most extensive evidence for safety-in-use.

Class II substances are simply intermediate. They are less clearly innocuous than those of class I, but do not offer the basis either of the positive indication of toxicity or of the lack of knowledge characteristic of those in class III.

A similar classification, but differently derived, has been used in the safety evaluation of flavourings (Oser & Hall, 1977). While the system outlined here is more broadly based, encompassing most ingested substances, the two classifications are wholly compatible.

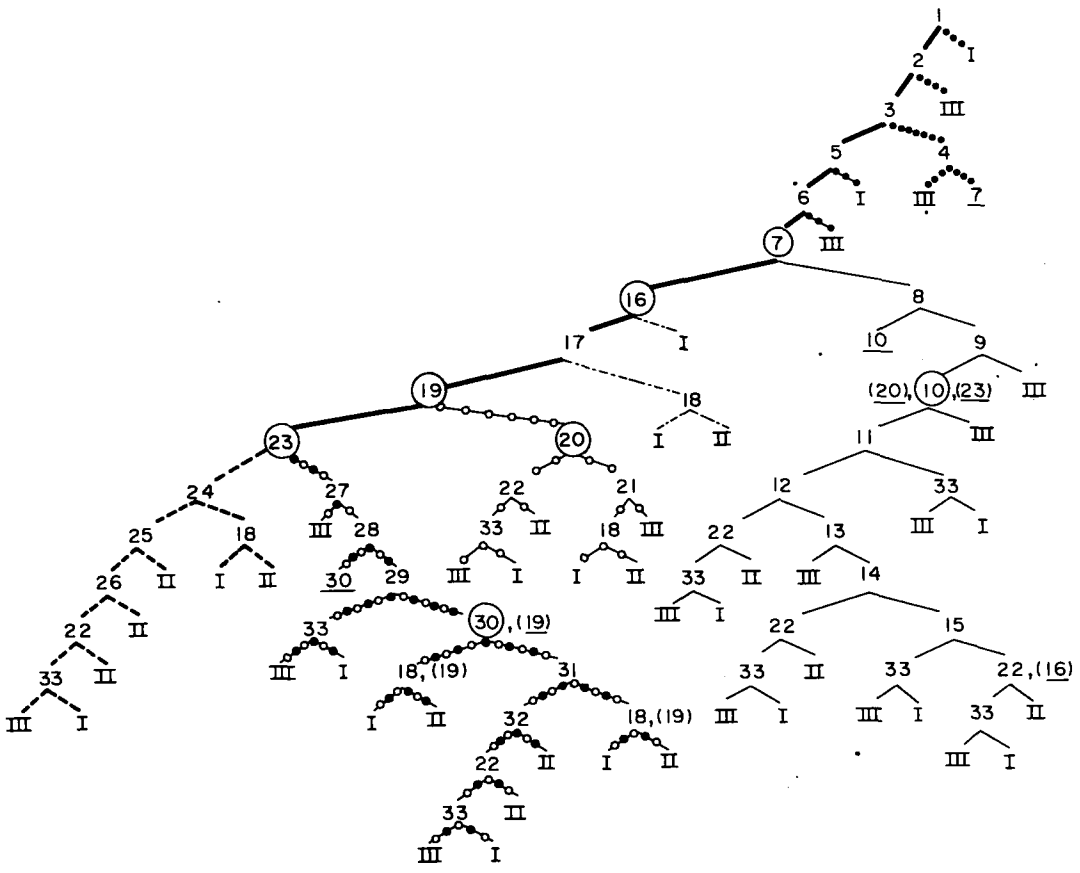


Fig. 1. A schematic diagram of a decision tree for the estimation of probable toxicity. Assessors should (a) start with question 1, (b) proceed by 'no' ↙ or ↘ 'yes', (c) move from any underscored number encountered to same circled number and (d) proceed to final classes I, II or III. Working downwards through the tree, the symbols designate the following groupings: biological normality (●●●), high and low toxicity (●–●–●); heterocyclics (——); terpenoids (–·–·); aliphatics (–O–O–O); aromatics (–O–●–O); alicyclics (– – –).

Definitions for use with the decision tree

The use of this decision tree and these definitions presupposes a working familiarity with organic chemistry, biochemistry and food chemistry. Even individuals with a broad knowledge in these areas will find it useful to consult references such as the *Merck Index* (1976) and the 'Weurman Report' (Central Institute for Nutrition and Food Research TNO, 1973), for detailed information on natural occurrence in food, and *Hawk's Physiological Chemistry* (Oser, 1965).

Users should review these definitions carefully and refer to them frequently. Because they are fashioned to serve the needs of this paper, they differ in several minor, but important, respects from the meanings commonly attributed to these words and phrases. Italicized key words used in the procedure (pp. 258–263) are followed by a letter, in parenthesis, referring to the following definitions:

(A) *Aliphatic* includes olefinic and polyolefinic, but not acetylenic or alicyclic compounds.

(B) *Aromatic* means that the substance has at least one benzene, furan, thiophene, pyridine or pyrrole ring, however substituted and whether or not it is fused to another ring.

(C) *Common component of food.* In something as diverse, changing and occasionally uncertain as natural occurrence, it is only possible to define a guideline, not a firm rule. For this decision tree, the term *common component of food* denotes a substance that has been reported in the recognized literature as occurring in significant quantity (approximately 50 ppm or more) in at least one major food, or in trace quantities at the ppm level or less in several foods, including minor or less frequently consumed foods. The latter include spices, herbs and ethnic specialities. This definition *excludes* natural or man-made contaminants, and hormones.

(D) *Common terpene* means an isoprenoid compound (carbon skeleton made up of two or more 5-carbon isoprene units), reported in the literature as a more than trace constituent of two or more generally consumed foods, either raw or as ordinarily prepared for consumption, without added ingredients.

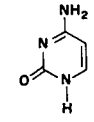
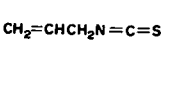
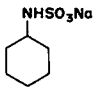
(E) *Functional group* is a portion (sometimes called a radical) of an organic molecule consisting of a combination of atoms of two or more elements (at least one of which is not hydrogen or carbon) and causing the molecule to exhibit a characteristic set of reactions. For the purpose of this classification this definition excludes carbon-carbon double bonds and aromatic rings.

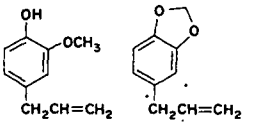
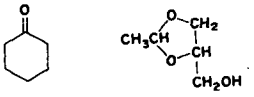
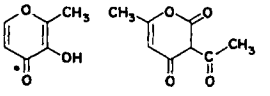
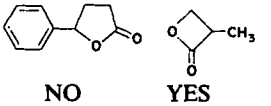
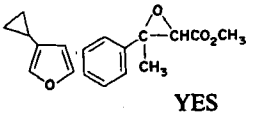
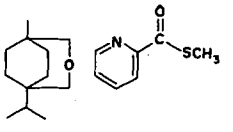
(F) *Normal constituent of the body* means any systemic constituent present at a normal physiological level, whether free or combined, except hormones. This includes essential nutrients and major food constituents and the physiologically normal metabolites of each. It excludes transitory substances present only as a result

of (a) trace constituents of food, (b) gut contents or (c) products of the actions of the gut flora.
(G) *Open chain* means the absence of any ring structure.
(H) *Readily hydrolysed* means known to be or, in the absence of any contra-indication based on structure, assumed to be hydrolysed either during food preparation or by physiological processes after consumption.
(I) *Simply branched* means branched at C–C bonds, with branches of two or more C atoms, at not more than two points along the main chain, with no secondary branching. Multiple branching, consisting only of 1-carbon moieties, falls within this definition of simply branched.
(J) *Sterically hindered* means posing steric hindrance to a functional group equivalent to or greater than that exhibited by *o-tert*-butyl or 2,6-disubstitution on an aromatic ring.
(K) *Structurally closely related* means
(a) a member, not more than two carbon atoms removed, in a homologous series, except ethoxy and higher homologues of a methoxy compound;
(b) a primary alcohol and its aldehyde, or either of these and the corresponding carboxylic acid;
(c) a secondary alcohol and the corresponding ketone;
(d) structural isomers unlikely to involve steric hindrance of a functional group;
(e) compounds with the same functional groups, the remainder of the compound being known to be easily and harmlessly metabolized;
(f) non-sterically hindered esters, thioesters, acetals, ketals and their components.

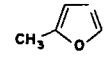
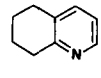
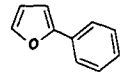
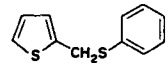
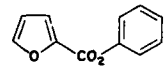
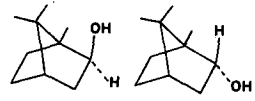
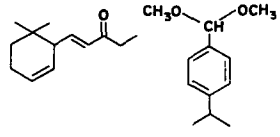
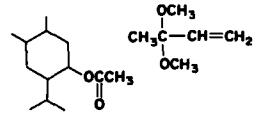
Procedure for the decision tree

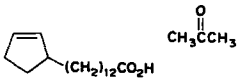
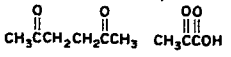
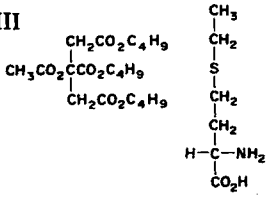
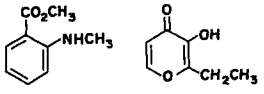
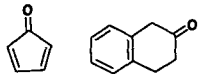
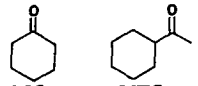
In the light of the chemical structure of the substance and using the accompanying definitions, answer the following questions, moving in the order indicated by the question numbers in the 'no' or 'yes' columns until a classification—I, II, or III—is reached. In borderline cases, choose II in preference to I, and III in preference to II. In cases where a structure is subdivided into individual residues, classify the whole structure according to the most conservative classification of the separate residues. In treating functional groups, consider the entire group, not the individual fragments (e.g. a thioamide, $RCSNH_2$, is a *thioamide* not a thione and an amine; a hydrazine, $RNHNH_2$, is not two amines; an anhydride is not an ether and two vicinal ketones).

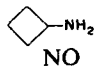
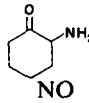
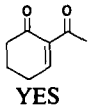
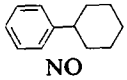
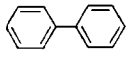
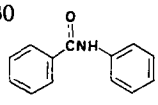
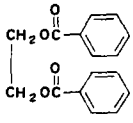
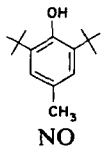
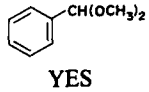
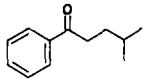
	If 'no'	If 'yes'	Examples with answers (and following step or classification)
	....proceed to....		
1. Is the substance a *normal constituent of the body* (F) or an optical isomer of such? This question throws into class I all normal constituents of body tissues and fluids, including normal metabolites. Hormones are excluded, as are, by implication, the metabolites of environmental and food contaminants or those resulting from disease states.	2	I	 NO (2)  YES (Class I)
2. Does the substance contain any of the following functional groups: an *aliphatic* (A) secondary amine or a salt thereof, cyano, *N*-nitroso, diazo (e.g. CH_2N_2), triazeno ($RN{=}NNH_2$) or quaternary nitrogen, except in any of the following forms: $>C{=}N^+R_2$, $>C{=}N^+H_2$ or the hydrochloride or sulphate salt of a primary or tertiary amine? Questions 2, 3 and 4 are a means of placing in class III those structures that contain elements or valency states often associated with enhanced toxicity. Halo-, nitro-, *N*-nitroso- and diazo- compounds, organophosphates, quaternary nitrogen compounds (although note modifying comment), and similar xenobiotic structures should cause 'yes' answers to questions 2 and 3 and a 'no' answer to question 4.	3	III	 NO (3)  YES (Class III)
3. Does the structure contain elements other than carbon, hydrogen, oxygen, nitrogen or divalent sulphur?	5	4	 NO (5)  YES (4)
4. Do all elements not listed in question 3 occur only as (a) a sodium, potassium, calcium, magnesium or ammonium salt of a carboxylic acid, or (b) a sulphate or hydrochloride of an amine, or (c) a sodium, potassium or calcium sulphonate, sulphamate or sulphate? (If the answer is yes, treat as the free acid, amine, unsulphonated or unsulphated compound, except for the purposes of questions 24 and 33, and proceed.) This is intended to let through, for further consideration, certain acid, amine, sulphonate and sulphate salts. Sulphamate salts are treated as such because they are not readily hydrolysed.	III	7	$CHCl_3$ NO (Class III)  YES (7)

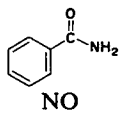
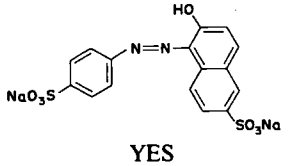
	If 'no' ....proceed to....	If 'yes'	Examples with answers
5. Is it a *simply branched* (I) acyclic *aliphatic* (A) hydrocarbon or a common carbohydrate? This drops out the generally innocuous hydrocarbons and carbohydrates.	6	I	 Xylose NO (6) YES (Class I)
6. Is the substance a benzene derivative bearing substituents consisting *only* of (a) hydrocarbon chains or 1'-hydroxy or hydroxy ester-substituted hydrocarbon chains and (b) one or more alkoxy groups, one of which must be para to the hydrocarbon chain in (a)? This places in class III safrole. myristicin and related substances.	7	III	 NO (7) YES (Class III)
7. Is the substance heterocyclic?	16	8	 NO (16) YES (8)
8. Is it a lactone or cyclic diester? This question separates the lactones and cyclic diesters from other heterocyclic compounds.	10	9	 NO (10) YES (9)
9. Is it a lactone fused to another ring, or a five- or six-membered α,β-unsaturated lactone? This places certain lactones known or suspected to be of unusual toxicity in class III.	*	III	 NO (23) YES (Class III)
10. Is it a 3-membered heterocycle? This places such substances as the epoxides and ethylenimine in class III.	11	III	 NO (11) YES (Class III)
11. Disregarding only the heteroatoms in any one ring, does that heterocyclic ring contain or bear substituents other than *simply branched* (I) hydrocarbons (including bridged chains and monocyclic aryl or alkyl structures), alkyl alcohols, aldehydes, acetals, ketones, ketals, acids, esters (including cyclic esters other than lactones), mercaptans, sulphides, methyl ethers, hydroxy or single rings (hetero or aryl) with no substituents other than those just listed? Questions 11–15 separate out various categories of heteroaromatic substances. Under 11, set aside and do not consider the atom(s), usually oxygen, nitrogen or sulphur, making the ring heterocyclic. If there is more than one hetero ring, regard each ring separately, with the remainder of the structure as substituents of that hetero ring. Other than the heterocyclic atom(s), does the ring carry anything besides the simple groups listed? If so, the answer is 'yes'	12	33	 NO (12) YES (33)

*If it is a lactone, from this point on treat the structure as if it were the hydroxy acid in the form of its more stable tautomer and proceed to question 20 if it is open chain, to 10 if it is heterocyclic and to 23 if it is carbocyclic; if it is a cyclic diester treat as the separate components.

	If 'no' ....proceed to....	If 'yes' ....proceed to....	Examples with answers
and the next question 33. If not, then classify further by question 12 *et seq.* Bridged-chain derivatives may be represented by structures like the bicyclic ether 1,4-cineole while monocyclic aryl derivatives may be represented by compounds like benzaldehyde propylene glycol acetal or 3-phenyl-2-furancarboxaldehyde.			
12. Is it hetero*aromatic* (B)? This question separates the aromatic heterocyclics for the purpose of considering whether they are polynuclear (question 14) or unsubstituted (question 13).	22	13	 NO (22)  YES (13)
13. Does the ring bear any substituents?	III	14	 NO (Class III)  YES (14)
14. Does the structure contain more than one *aromatic* (B) ring?	22	15	 NO (22)  YES (15)
15. Is it *readily hydrolysed* (H) to mononuclear residues? (If yes, treat the mononuclear heterocyclic residues by question 22 and any carbocyclic residue by question 16.)	33	22	  NO (33) YES (22 & 16)
16. Is it a *common terpene* (D)-hydrocarbon, -alcohol, -aldehyde or -carboxylic acid (not a ketone)? Questions 16 and 17 deal with terpenes. A hydrocarbon terpene that is a *common terpene* (D) and has not already been put in class I by question 5, would go into class I by question 16.	17	I	 NO (17) YES (Class I)
17. Is the substance *readily hydrolysed* (H) to a *common terpene* (D), -alcohol, -aldehyde or -carboxylic acid? (If the answer is yes, treat the hydrolysed residues separately and proceed to 18 for the terpene moiety and to 19 for any non-terpenoid moiety.) Since there may be substances that are hydrolysed to two or more residues, one of which is a terpene, treat the residues separately from question 18 onward to conclusion.	19	18	 NO (19) YES (18 & 19)
18. Is the substance one of the following: (a) a vicinal diketone; or a ketone or ketal of a ketone attached to a terminal vinyl group (b) a secondary alcohol or ester of a secondary alcohol attached to a terminal vinyl group (c) allyl alcohol or its acetal, ketal or ester derivative (d) allyl mercaptan, an allyl sulphide, an allyl thioester or allyl amine (e) acrolein, a methacrolein or their acetals (f) acrylic or methacrylic acid (g) an acetylenic compound (h) an acyclic *aliphatic* (A) ketone, ketal or ketoalcohol with no other functional groups and with four or more carbons on either side of the keto group (i) a substance in which the *functional groups* (E) are all *sterically hindered* (J).	I	II	 NO (Class I) YES (Class II)

	If 'no' ....proceed to....	If 'yes'	Examples with answers
Question 18 examines the terpenes (and later the open-chain and mononuclear substances by reference) to determine whether they contain certain structural features generally thought to be associated with some enhanced toxicity.			
19. Is the substance *open chain* (G)? Questions 19–21 deal with open-chain substances.	23	20	 NO (23) YES (20)
20. Is the structure a linear or *simply branched* (I) *aliphatic* (A) compound containing any one or combination of only the following *functional groups* (E): (a) four or less, each, of alcohol, aldehyde, carboxylic acid or esters and/or (b) one each of one or more of the following: acetal, either ketone or ketal but not both, mercaptan, sulphide (mono- or poly-), thioester, polyoxyethylene [(—OCH_2CH_2—)$_x$ with x no greater than 4], or primary or tertiary amine? This question should be answered 'yes' if the structure contains one or any possible combination of alcoholic, aldehydic or carboxylic acid or ester groups, provided there are no more than four of any one kind. It should be answered 'yes' if the structure contains in addition to, or instead of, those just listed, any assortment of no more than one each of the following: acetal, either ketone or ketal but not both, mercaptan, mono- or polysulphide, thioester, polyoxyethylene, primary or tertiary amine. Answer the question 'no' if the structure contains more than four of any of the first set of groups, more than one of the second set, or any substituent not listed.	22	21	 NO (22) YES (21)
21. Does the structure contain three or more different types of functional groups (exclude methoxy and consider acids and esters as one functional type)? *Aliphatic* (A) compounds containing three or more different functional groups (excluding methoxy) are too complex to permit satisfactory prediction of toxicity. They should go, therefore, into class III. However, we do not wish to put into class III polyesters and similar substances, so these and the methoxy compounds get passed along to question 18.	18	III	 NO (18) YES (Class III)
22. Is the substance a *common component of food* (C) or *structurally closely related* (K) to a *common component of food* (C)? This question places in class II the natural, nature-identical and nearly nature-identical substances not already put into class I by physiological occurrence or structural criteria. An artificial (i.e. non-nature-identical) substance, or one not closely related, goes to question 33.	33	II	 NO (33) YES (Class II)
23. Is the substance *aromatic* (B)? Questions 23–26 deal with alicylic substances.	24	27	 NO (24) YES (27)
24. Is the substance monocarbocyclic (excluding cyclopropane or cyclobutane and their derivatives) with ring or *aliphatic* (A) side chains, unsubstituted or containing only alcohol, aldehyde, side-chain ketone, acid, ester, or sodium, potassium or calcium sulphonate or sulphamate, or acyclic acetal or ketal?	25	18	 NO (25) YES (18)

	If 'no' ....proceed to....	If 'yes'	Examples with answers
25. Is the substance (a) a cyclopropane or cyclobutane with only the substituents mentioned in question 24 or (b) a mono- or bicyclic sulphide or mercaptan?	26	II	 NO (26)  YES (Class II)
26. Does the structure contain no functional groups other than those listed in question 24 and is it either a monocyloalkanone or a bicyclic compound with or without a ring ketone?	22	II	 NO (22)  YES (Class II)
27. Does (do) the ring(s) have any substituents? Questions 27–31 deal with aromatic compounds.	III	28	 NO (Class III)  YES (28)
28. Does the structure contain more than one *aromatic* (B) ring?	30	29	 NO (30)  YES (29)
29. Is it *readily hydrolysed* (H) to mononuclear residues? (If yes treat the individual aromatic mononuclear residues by question 30 and any other residue by question 19.)	33	30	 NO (33)  YES (30 & 19)
30. Disregarding ring hydroxy or methoxy does the ring bear substituents *other* than 1–5-carbon *aliphatic* (A) groups, either hydrocarbon or containing alcohol, ketone, aldehyde, carboxyl or simple esters that may be hydrolysed to ring substituents of five or less carbons? (If a simple ester that may be hydrolysed, treat the aromatic portion by question 18 and the residue by question 19.) This should be answered 'no' if the ring bears only aliphatic groups of five carbons or less, which are either hydrocarbon in nature or contain the groups listed. If the ring bears any other substituents than those listed, the question should be answered 'yes' and one should proceed to question 31.	18	31	 NO (18)  YES (31)
31. Is the substance an acyclic acetal, -ketal or -ester of any of the above substances (see question 30)? (If yes, assume hydrolysis and treat the non-aromatic residues by question 19 and. the aromatic residue by question 18.) This question is simply designed to see whether the substance would fit within the definition of question 30 if it were not an acetal, a ketal or an ester. In other words, would the substance carry only the groups listed in question 30.	32	18	 NO (32)  YES (18)
32. Does the substance contain only the *functional groups* (E) listed in question 30, or their derivatives listed in question 31, but with any or all of the following: (a) a single fused non-aromatic carbocyclic ring, (b) *aliphatic* (A) substituent chains longer than five carbon atoms, or (c) a polyoxyethylene [(—OCH_2CH_2—)$_x$ with x no greater than 4] chain either on the aromatic ring or on an *aliphatic* (A) side chain? Part (a) is intended to allow simple derivatives of tetralin into class II while putting polycyclic compounds such as the steroids ultimately into class III except those that may be normal food	22	II	 NO (22)  YES (Class II)

	If 'no'	If 'yes'	Examples with answers
	....proceed to....		
components. Part (b) allows compounds with permitted functional groups but longer side chains into class II instead of sending them eventually into class III. Part (c) puts short-chain polyoxyethylene derivatives of aryl compounds into class II rather than class III.			
33. Does the substance bear on every major structural component at least one sodium, potassium, or calcium sulphonate or sulphamate for every 20 or fewer carbon atoms without any free primary amines except those adjacent to the sulphonate or sulphamate. Sodium, potassium, and calcium sulphonate and sulphamate salts have a strong tendency to decrease toxicity by promoting solubility and rapid excretion. This is particularly noticeable, for example, with some of the food colourings. It is important that the substance bears sufficient sulphonate groups, including one on each of the major structural fragments into which the original compound might be metabolized. This question serves to steer sulphonated compounds except those with amines non-adjacent to the sulphonate into a presumptively less toxic classification than the compounds would occupy if unsulphonated.	III	I	 NO (Class III)  YES (Class I)

Discussion

The decision tree

The questions that comprise the decision tree are a conscious compromise between discrimination and complexity. It will doubtless occur to many readers to suggest certain additional questions or changes of wording which would permit refinement and a clearer separation between the three classes. At the same time, we have tried to keep the questions as simple and few in number as possible. Further improvement is undoubtedly possible, and we welcome suggestions to that end.

We wish it were possible to make this whole effort more simple, but the task involves many simultaneous considerations which render it inherently complex. Undoubtedly we have not yet found ideally clear wording for many of the questions, but this represents the result of extensive trials by many workers.

Those who wish to use the decision tree should not be deterred by its initial complexity or by the slowness of the first attempts to use it. We have found that, with only a little training, equivalent to a careful reading of this paper, and a short period of practice, anyone with a background of moderate organic or biochemical knowledge can apply the decision tree with few errors and little difficulty. Reliability is enhanced when two or more individuals use it separately and compare their results. In practice, the tree contains a fair amount of redundancy. We have observed that in most instances an 'incorrect' answer leads, nevertheless, to the proper final classification. It should be stressed again that the decision tree is not intended to be used alone. It must be combined with information on present or probable intakes covered later in this discussion for an estimate of the implied hazard to be developed.

We have attempted several general tests of the utility of this decision tree. First, we have sought to classify, regardless of the availability of detailed toxicological data, a number of substances, some relatively innocuous and others highly toxic, to see if they fell into the appropriate classes. A host of substances known or presumed to be quite innocuous fell into class I. As far as we have been able to test, the recognized carcinogens, natural toxicants and pharmacologically potent drugs fall, as they should, in class III.

We are grateful to Dr. Marvin Legator and Mr. Stephen Rinkus for providing us with a list of 247 substances reported to cause cancer in two or more species. This was compiled from The Registry of Toxic Effects of Chemical Substances (Department of Health, Education, and Welfare, 1976). Twenty of these substances were polymers or inorganic materials to which this approach is not applicable. Of the remaining 227, all fall into class III with the single exception of xanthine, which falls into class I. Xanthine is a normal purine metabolite and an endogenous constituent of many human and animal tissues. The tests in which it has been reported to produce neoplasms involved not the oral route but subcutaneous administration and implantation. Thus we do not regard its classification as an aberrant result of this decision tree.

We have also applied a more critical evaluation by tabulating by class (I, II or III) the no-observed-adverse-effect levels (hereafter termed no-effect levels) for a number of food additives, drugs, industrial chemicals and pesticides. We have used the no-effect levels derived from relevant recent literature based on either short-term or chronic studies. Where these levels were reported in terms of dietary concentration, we used commonly accepted factors to convert to mg/kg body weight. In virtually all cases except those substances with no-effect levels above 500 mg/kg body weight/day, we have restricted the tabulation to toxicity tests in which the next higher feeding level above the no-effect level (i.e. the lowest level to show some adverse effect) was no more than five times the no-effect level. While this restraint rules out much of the

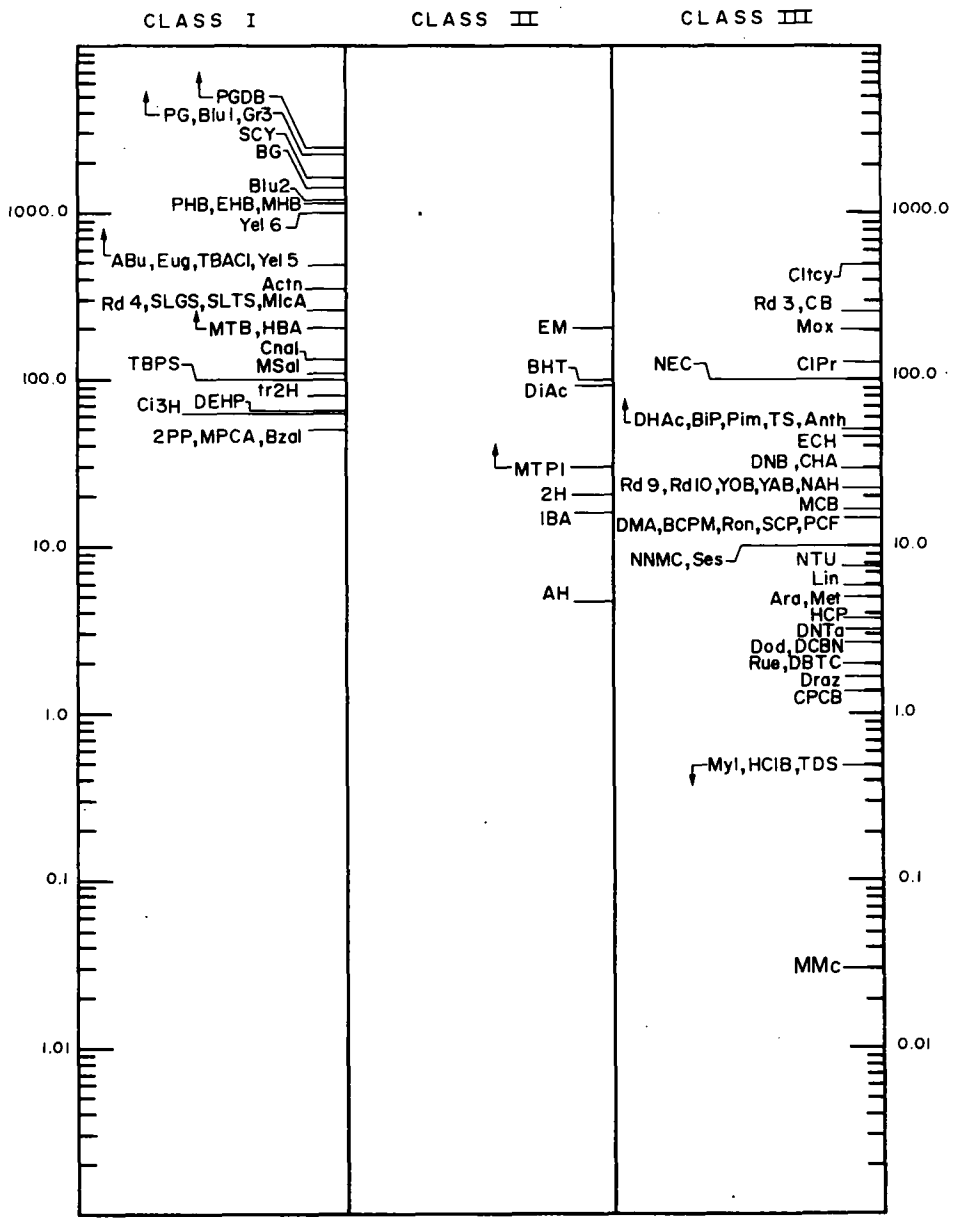


Fig. 2. Rank ordering of no-observed-adverse-effect levels within each class. Arrows indicate no-effect-levels greater or less than those shown; abbreviations refer to the substances listed in Table 1.

available toxicological data, it removes 'fuzziness' and provides a cleaner separation between classes than would otherwise occur.

With minor exceptions we have included without editing all data that have so far come to our attention, and that meet our criteria. Exceptions involved essentially duplicative data, one instance of the use of too few animals, and two cases in which the authors had chosen a lower no-effect level largely because even that provided an ample margin of safety.

The substances were run through the decision tree to determine the class into which they fell and then ranked by no-effect level within each class. The results of this tabulation are shown in Fig. 2 and Appendix 1 (p. 267). The appendix and the figure can be cross-related by the compound abbreviations indicated. Appendix 1 lists the abbreviation, common name, no-effect level and literature reference for the no-effect level. For the convenience of those who may wish to check their own use of the decision tree, the appendix also shows the 'track' for each substance through the tree.

It has been our general intent that the most toxic substance in class I (i.e. that with the lowest no-effect level) should have a no-effect level at or above 50 mg/kg body weight/day. With a hundredfold safety factor, this corresponds approximately to an intake of 25 mg/day by a human adult—a fairly substantial intake. Few exogenous chemicals that are not nutrients are consumed in larger amounts. It may well be that substances we have not sought out from the literature and plotted would fall into class I below

this level. This would raise the question of whether we wished to accept that state of affairs or, more probably, revise the decision tree structure so as to reclassify them into class II or class III.

Two facts stand out from the tabulation in Fig. 2. The first is the fact that the decision tree does result in a fairly clear-cut separation. All of the really innocuous substances fall into class I. All of the highly toxic compounds are in class III. Class II is both in between and gratifyingly smaller. We should be concerned if most substances wound up indecisively in class II.

The second obvious conclusion is that there is a slight overlap between the classes, particularly in the 10–100 mg/kg body weight area. This may seem to be a disadvantage, but on reflection, it is clear that this must be so. Substances much more innocuous than 1000 mg/kg body weight/day are so patently harmless that it is rare to find them adequately studied to the point of establishing no-effect levels. Similarly, substances much more toxic than 1 mg/kg body weight/day are so toxic that unless they are useful economic poisons, drugs or chemical warfare agents, they are unlikely to be used, and the data on them are unlikely to be published. Thus, the area roughly between 10 and 100 mg and to some extent on either side of it, is simply where the data base lies. These are the substances that are worth testing for reasons of utility and that require testing for reasons of probable hazard. Furthermore, inspection of the class III substances with no-effect levels of 50 mg/kg body weight or more indicates that virtually none of them could be judged in advance to be innocuous. They are 'unexpectedly nontoxic', and therefore merit retention in class III.

Estimates of presumable risk

To obtain an estimate of presumable risk, for setting priorities and for the preliminary establishment of criteria, one may combine in a grid, the estimate of toxic threat from the decision tree with data on intake (Table 1). We have utilized here daily per capita intake, mindful of its limitations and of the desirability—even in preliminary estimates—of taking into account the range of intakes across the population (Hall, Kahan, Merwin, Wharton, Dodger & Abrams, 1978).

In every box under each intake bracket for classes I and II is a figure derived from the presumptive, but conservative, no-effect level using the most toxic (lowest no-effect) level in that class (50 mg/kg for class I and 5 mg/kg for class III; see Fig. 2 and Appendix 1). That 'presumptive no-effect level' is divided by the upper limit of intake for that bracket, to obtain what we shall call the 'protection index' (PI). For example, the lowest no-effect level' for any class I substance is 50 mg/kg body weight, equivalent to an intake of 2500 mg for a 50-kg adult; if the *per capita* daily intake of such a substance is between 1 and 10 μg (10^{-3}–10^{-2} mg) dividing 2500 mg by the top of that intake bracket (10 μg) gives a PI of 250,000 (Table 1). The other figures for classes I and II are similarly derived. No such figure can be adduced for class III, since there is not necessarily any definable lower limit of effect for this class. If we were to assume, however, that few if any ingested substances would have a no-effect level lower than 0·1 mg/kg equivalent to an intake of 5 mg/day, an intake of 0·1 μg/day would imply a PI of approximately 50,000. In this grid, the substances identified, on the basis of these presumptions as most hazardous (those with the lowest PI) are in the lower right and those identified as most safe are in the upper left.

The reader should note that we do not suggest that the actual 'safety factor' for a particular class I substance consumed at a designated intake is the same as the PI shown in Table 1. This is most obviously true at the higher intakes and higher presumptive toxicities. Only actual testing will establish a no-effect level from which a 'safety factor' can be deduced. The distribution of values within the different classes in Appendix 1 suggests that the experimentally determined no-effect level and the resulting safety factor will usually be at least an order of magnitude higher than the lower limit for the class assumed in Table 1.

We do not, then, assume that these PI values are accurate; indeed they are probably quite conservative. But they do provide an 'index of suspicion', by which to focus attention in an orderly way on the apparently greater hazards and to consign to low priority the very remote risks. Moreover, they provide a basis for a preliminary specification of the kinds of information appropriate for a demonstration of safety for a substance with that priority. We suggest four such categories, designated A–D.

The letter 'A' applied to PI values of 250,000 and higher represents the lowest priority. Unless other considerations intrude, priority A requires physical

Table 1. *Classification by presumable risk showing 'protection index' (PI) and categories of safety*

	PI* and category of safety for a daily per capita intake (in mg) of							
Class	$<10^{-5}$	10^{-5}–10^{-4}	10^{-4}–10^{-3}	10^{-3}–10^{-2}	10^{-2}–10^{-1}	0·1–1·0	1·0–10	>10
I	>250,000,000 A	>25,000,000 A	>2,500,000 A	>250,000 A	>25,000 B	>2500 C	>250 C	<250 D
II	>25,000,000 A	>2,500,000 A	>250,000 A	>25,000 B	>2500 C	>250 C	>25 D	<25 D
III	(500,000) A	(50,000) B	(5000) C	(500) C	(50) D	(?) D	(?) D	(?) D

$$*PI = \frac{\text{lowest no-effect level for class (mg/kg body weight)} \times 50 \text{ (kg body weight)}}{\text{maximum of intake range (mg) in column}}.$$

and chemical data for each substance, but denotes no present need for the acquisition of actual animal data, at least for direct and most indirect additives. Certain types of simple screening tests may be applied when and if their reproducibility and correlation with feeding studies are adequately validated by sufficient collaborative testing on a broad variety of food constituents and contaminants.

'B' and 'C' represent progressively higher priority for more intensive investigation. While a variety of trade-offs may be contemplated in these intermediate categories among various kinds and quantities of data, both imply the need for animal data, and 'C' would ordinarily imply at least sub-chronic data plus other evidence of safe metabolic disposition. 'D' denotes the necessity for chronic studies, plus, where appropriate, other supporting data.

It should be evident that other factors beyond those dwelt on here affect the direction and extent of testing. The importance (irreplaceability) of a substance, the nature of the anticipated toxic effect, the level of organizational or social concern, the sensitivity of particular groups of users, more refined data on the distribution of exposure and, not least, the 'gut feeling' of the professional toxicologist must be involved. These additional factors should be of greater influence with respect to class III substances, where the PI is less clear and the anticipated adverse effects may range more widely and be more serious. The structure we suggest here is a guideline, not a channel. Even more importantly, we stress again that this is intended only as a guide to the acquisition of data, not as a substitute for data. When actual tests results are available they must weigh heavily in determining the direction and extent of further testing.

Finally, this procedure requires knowledge of chemical structure and reasonably accurate estimates of intake. Where either is lacking, this method of estimating hazard *must not* be applied. If structure is unknown, the decision tree cannot be used, and one must not assume that a substance of unknown structure is necessarily in class III. If intake is unknown, a most critical factor in determining hazard is absent. One cannot use the lower limit of sensitivity of an analytical method as the basis for an assumed intake in this approach. The method cannot be applied where positive analytical results or estimates of intake based on actual use in food are lacking.

We welcome application, criticism and improvement of the 'decision tree' as a tool, and of this approach to establishing priorities and criteria. We venture to believe that it has some general usefulness in helping to make a number of the thousands of decisions that lie ahead.

Acknowledgements—The preparation of this paper has been made possible only through the advice and assistance of a remarkably large number of people. Dr. Bernard L. Oser has provided encouragement, insight and suggestions, as have Drs Lauren A. Woods, Howard C. Spencer, David W. Fassett, Anthony M. Ambrose and R. Tecwyn Williams. Drs Otho D. Easterday and John C. Kirschman have given every possible assistance. Drs John A. Zapp, Jr., Robert A. Scala, Jessie M. Norris and John P. Frawley and Mr. Carrol S. Weil have been generous with their time and thought. Dr. Jan Stofberg and members of the Food Additives Committee of the Flavor & Extract Manufacturers' Association rendered invaluable assistance in proving and improving the decision tree by processing through it hundreds of different substances. Drs William G. Galetto and William N. Zeiger, Mr. Earl J. Merwin and Mrs. Merle I. Eiss did most of the literature search for toxicological data and provided the necessary structural information, including some corrections to the literature, from their formidable knowledge of organic nomenclature. Finally, many others in government, academia and industry have provided encouragement and advice.

REFERENCES

Arthur D. Little, Inc. (1977). Pre-screening for Environmental Hazards—A System for Selecting and Prioritizing Chemicals. Prepared for Environmental Protection Agency, Office of Toxic Substances, Washington, DC. PB-267 093. US Department of Commerce, National Technical Information Service, Springfield, VA.

Central Institute for Nutrition and Food Research TNO (1973). Lists of Volatile Compounds in Food. Supplements 1–7, 1975. Zeist, The Netherlands.

Food and Drug Administration Bureau of Foods (1977). FDA Compliance Program Evaluation, FY '74 Total Diet Studies (7320.08). 21 January. Department of Health, Education and Welfare, Washington, DC.

Food Protection Committee (1970). Evaluating the Safety of Food Chemicals. National Academy of Sciences–National Research Council, Washington, DC.

Hall, R. L., Kahan, G., Merwin, E. J., Wharton, I. A., Dodger, D. F. & Abrams, I. J. (1978). Estimating Distribution of Daily Intakes of Certain GRAS Substances. In preparation for Committee on GRAS List Survey, Phase III. National Academy of Sciences–National Research Council, Washington, DC.

Joint FAO/WHO Expert Committee on Food Additives—Twentieth Report (1976). Evaluation of Certain Food Additives. *Tech. Rep. Ser. Wld Hlth Org.* **599**, p. 20.

Merck Index (1976). *An Encyclopedia of Chemicals and Drugs*. 9th Ed. Merck & Co. Inc., Rahway, NJ.

National Institute for Occupational Safety and Health (1976). The Registry of Toxic Effects of Chemical Substances. HEW Publication No. (NIOSH) 76–191. Department of Health, Education and Welfare, Washington, DC.

Oser, B. L. [Ed.] (1965). *Hawk's Physiological Chemistry*. 14th Ed. McGraw-Hill Book Co., New York.

Oser, B. L. & Hall, R. L. (1977). Criteria employed by the Expert Panel of FEMA for the GRAS evaluation of flavouring substances. *Fd Cosmet. Toxicol.* **15**, 457.

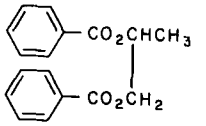
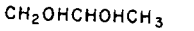
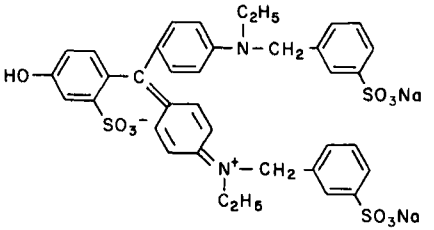
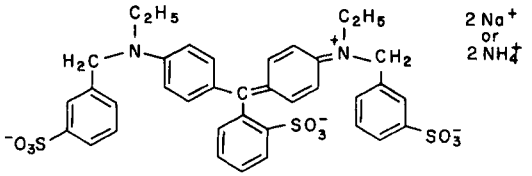
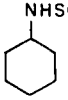
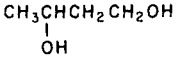
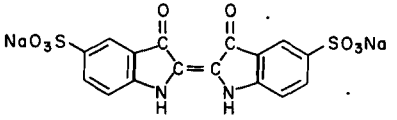
Panel on Chemicals and Health of the President's Science Advisory Committee (1973). Chemicals and Health. Report of the Panel on Chemicals and Health. p. 20. National Science Foundation, Washington, DC.

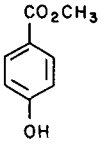
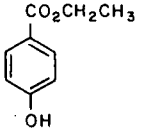
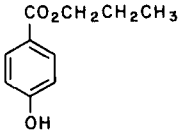
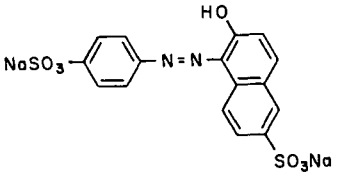
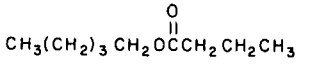
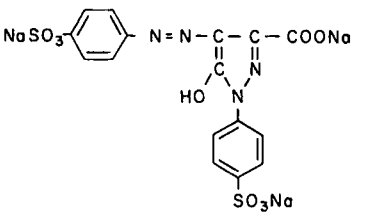
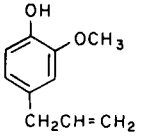
Price, D. & Desolla, J. (1969). *Little Science, Big Science*. p. 78. Columbia University Press, New York.

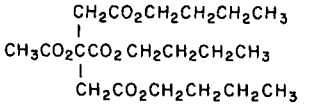
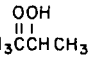
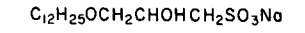
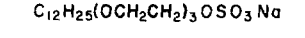
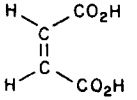
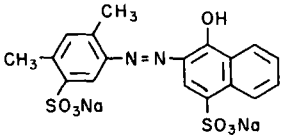
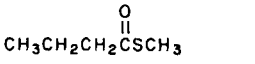
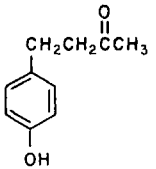
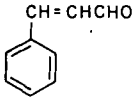
Select Committee on Flavor Evaluation Criteria (1976). Criteria for Evaluation of the Health Aspects of Using Flavoring Substances as Food Ingredients. Federation of American Societies for Experimental Biology, Life Sciences Research Office, Bethesda, MD.

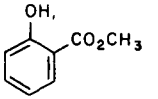
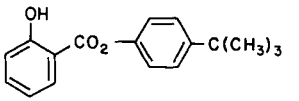
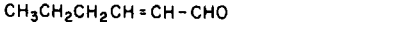
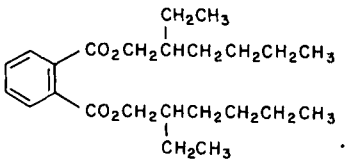
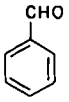
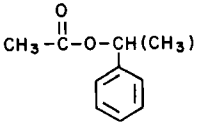
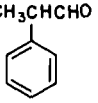
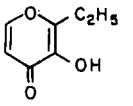
Subcommittee on Review of the GRAS List—Phase II (1972). A Comprehensive Survey of Industry on the Use of Food Chemicals Generally Recognized as Safe (Gras). Committee on Food Protection, Food and Nutrition Board, Division of Biology and Agriculture. National Academy of Sciences–National Research Council, Washington, DC.

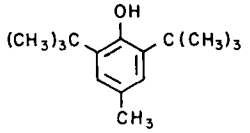
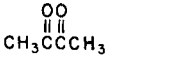
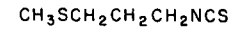
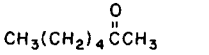
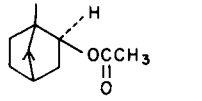
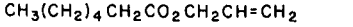
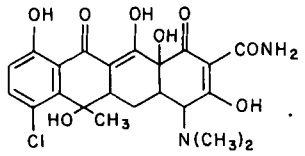
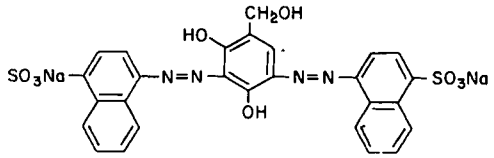
Appendix 1. *Tabulation of substances by class in order of decreasing no-observed-adverse-effect level*

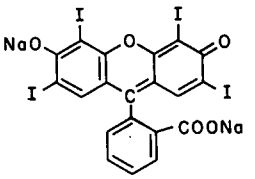
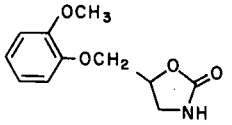
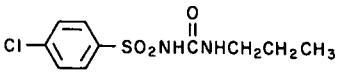
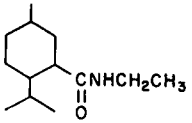
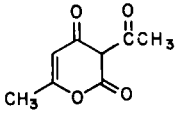
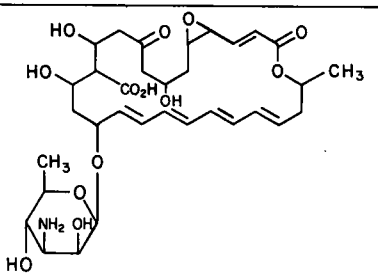
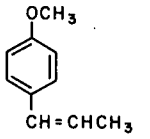
Abbreviation	Compound and track through decision tree (with reference to NEL in parenthesis)	No-effect level (mg/kg body weight)	Structure
	CLASS I		
PGDB	propylene glycol dibenzoate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29Y, $^{19Y,20Y,21N,18N-I}_{30N,18N-I}$ (GRAS submission)	>2541	
PG	Propylene glycol 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I Gaunt *et al. Fd Cosmet. Toxicol.* 1972, **10**, 151)	>2500	
Gr3	FD & C Green No. 3 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33Y—I (Hansen *et al. Fd Cosmet. Toxicol.* 1966, **4**, 389)	2500	
Blu1	FD & C Blue No.1 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33Y—I (Hansen *et al. Toxic. appl. Pharmac.* 1966, **8**, 29)	2500	
SCY	Sodium cyclamate 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19N, 23N, 24Y, 18N—I (Brantom *et al. Fd Cosmet. Toxicol.* 1973, **11**, 735)	1750	
BG	1,3-Butylene glycol 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (Scala *et al. Toxic. appl. Pharmac.* 1964, **6**, 358)	1500	
Blu2	FD & C Blue No. 2 (Indigo carmine) 1N, 2N, 3Y, 4Y, 7Y, 8N, 10N, 11Y, 33Y—I (Gaunt *et al. Fd Cosmet. Toxicol.* 1969, **7**, 17)	1350	

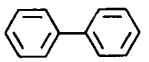
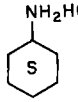
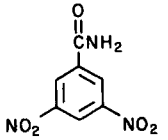
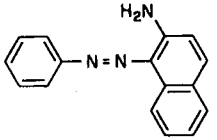
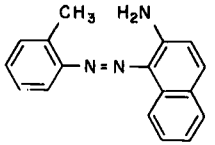
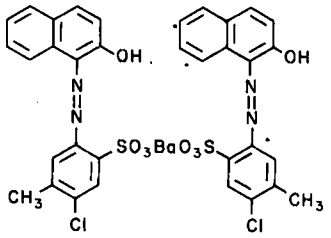
MHB	Methyl *p*-hydroxybenzoate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Matthews *et al. J. Am. pharm. Ass.* 1956, **45**, 260)	1200	
EHB	Ethyl *p*-hydroxybenzoate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Matthews *et al. J. Am. pharm. Ass.* 1956, **45**, 260)	1200	
PHB	Propyl *p*-hydroxybenzoate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Matthews *et al. J. Am. pharm. Ass.* 1956, **45**, 260)	1200	
Yel6	FD & C Yellow No. 6 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33Y—I (W. H. Hansen, unpublished data; cited from *Interbureau By-Lines* 1962, **3**, no. 3)	1000	
ABu	Amyl butyrate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (Hagan *et al. Fd Cosmet. Toxicol.* 1967, **5**, 141)	> 500	
Yel5	FD & C Yellow No. 5 1N, 2N, 3Y, 4Y, 7Y, 8N, 10N, 11Y, 33Y—I (Davis *et al. Toxic. appl. Pharmac.* 1964, **6**, 621)	500	
Eug	Eugenol 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Hagan *et al. Fd Cosmet. Toxicol.* 1967, **5**, 141)	500	

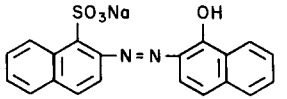
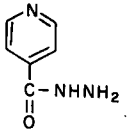
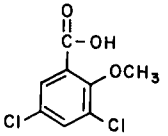
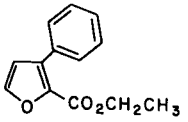
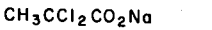
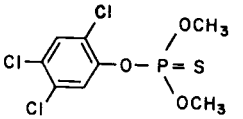
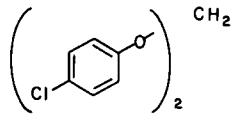
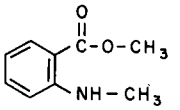
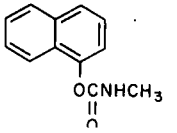
Abbreviation	Compound, track and reference	NEL (mg/kg)	Structure
TBACi	Tributyl acetylcitrate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (Finkelstein & Gold, *Toxic. appl. Pharmac.* 1959, **1**, 283)	500	
Actn	Acetoin 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (Gaunt *et al. Fd Cosmet. Toxicol.* 1972, **10**, 131)	330	
SLGS	Sodium lauryl glyceryl ether sulphonate 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19Y, 20N, 22N, 33Y—I (Tusing *et al. Toxic. appl, Pharmac.* 1962, **4**, 402)	250	
SLTS	Sodium lauryl trioxyethylene sulphate 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (Tusing *et al. Toxic. appl. Pharmac.* 1962, **4**, 402)	250	
MlcA	Maleic acid 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (Fitzhugh & Nelson, *J. Am. pharm. Ass.* 1947, **36**, 217)	250	
Rd4	FD & C Red No. 4 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33Y—I (Davis *et al. Toxic. appl. Pharmac.* 1966, **8**, 306)	250	
MTB	Methyl thiobutyrate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (GRAS submission)	>200	
HBA	*p*-Hydroxybenzyl acetone 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Gaunt *et al. Fd Cosmet. Toxicol.* 1970, **8**, 349)	200	
Cnal	Cinnamaldehyde 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Hagan *et al. Fd Cosmet. Toxicol.* 1967, **5**, 141)	125	

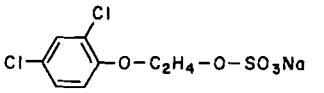
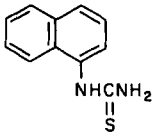
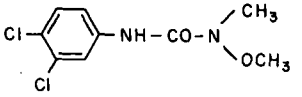
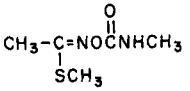
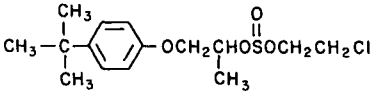
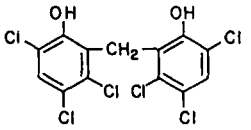
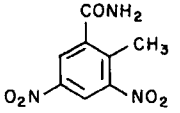
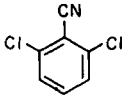
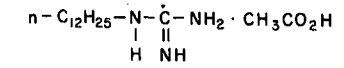
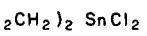
MSal	Methyl salicylate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Packman *et al. Pharmacologist* 1961, **3**, 62)	105	
TBPS	*tert*-Butylphenyl salicylate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29Y, 30N, 18N—I (Weil & McCollister, *J. agric. Fd Chem.* 1963, **11**, 486	100	
tr2H	*trans*-2-Hexenal 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (Gaunt *et al. Fd Cosmet. Toxicol.* 1971, **9**, 775)	80	
DEHP	Di-(2-ethylhexyl) phthalate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Weil & McCollister, *J. agric. Fd Chem.* 1963, **11**, 486)	65	
Ci3H	*cis*-3-Hexenol 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18N—I (Gaunt *et al. Fd Cosmet. Toxicol.* 1969, **7**, 451)	62·5	
Bzal	Benzaldehyde 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Hagan *et al. Fd Cosmet. Toxicol.* 1967, **5**, 141)	50	
MPCA	Methylphenylcarbinyl acetate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Gaunt *et al. Fd Cosmet. Toxicol.* 1974, **12**, 185)	50	
2PP	2-Phenylpropanal 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18N—I (Pelling *et al. Fd Cosmet. Toxicol.* 1976, **14**, 249)	50	
	CLASS II		
EM	Ethyl maltol 1N, 2N, 3N, 5N, 6N, 7Y, 8N, 10N, 11N, 12N, 22Y—II (Gralla *et al. Toxic. appl. Pharmac.* 1969, **15**, 604)	200	

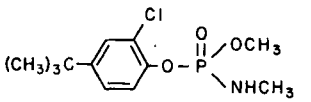
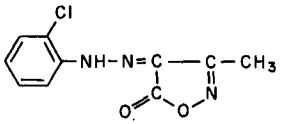
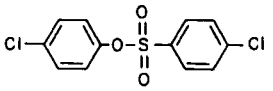
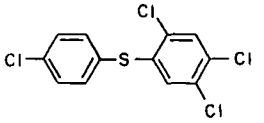
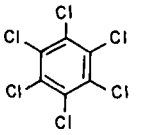
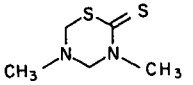
Abbreviation	Compound, track and reference	NEL (mg/kg)	Structure
BHT	Butylated hydroxytoluene 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30N, 18Y—II (NTIS Publ. PB-221 202, GRAS Food Ingredients)	100	
DiAc	Diacetyl 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20N, 22Y—II (Colley *et al. Fd Cosmet. Toxicol.* 1969, **7**, 571)	90	
MTPI	3-Methylthiopropyl isothiocyanate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20N, 22Y—II (GRAS submission)	>30	
2H	2-Heptanone 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18Y—II (Gaunt *et al. Fd Cosmet. Toxicol.* 1972, **10**, 625)	20	
IBA	Isobornylacetate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23N, 24N, 25N, 26N, 22Y—II (Gaunt *et al. Fd Cosmet. Toxicol.* 1971, **9**, 355)	15	
AH	Allyl heptanoate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20Y, 21N, 18Y—II (Hagan *et al. Toxic. appl. Pharmac.* 1965, **7**, 18; FDA File 997, 24 March 1964)	5	
	CLASS III		
Cltcy	Chlortetracycline 1N, 2N, 3Y, 4N—III (Dessau & Sullivan, *Toxic. appl. Pharmac.* 1961, **3**, 654)	500	
CB	Chocolate Brown HT 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33N—III (Hall *et al. Fd Cosmet. Toxicol.* 1966, **4**, 143)	250	

Rd3	FD & C Red No. 3 1N, 2N, 3Y, 4N—III (W. H. Hansen, unpublished data; cited from *Interbureau By-Lines* 1962, **3**, no. 3)	250	
Mox	Mephanoxalone 1N, 2N, 3N, 5N, 6N, 7Y, 8N, 10N, 11Y, 33N—III (Yeory *et al. Toxic. appl. Pharmac.* 1964, **6**, 642)	200	
ClPr	Chloropropamide 1N, 2N, 3Y, 4N—III (Delahunt *et al. Toxic. appl. Pharmac.* 1960, **2**, 195)	125	
NEC	*N*-Ethyl-2-isopropyl-5-methylcyclohexanecarboxamide 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23N, 24N, 25N, 26N, 22N, 33N—III (GRAS submission)	100	
DHAc	Dehydroacetic acid 1N, 2N, 3N, 5N, 6N, 7Y, 8Y, 9N, 20N, 22N, 33N—III (Weil & McCollister, *J. agric. Fd Chem.* 1963, **11**, 486)	>50	
Pim	Pimaricin 1N, 2N, 3N, 5N, 6N, 7Y, 8N, 10Y—III (Levinskas *et al. Toxic. appl. Pharmac.* 1966, **8**, 97)	50	
Anth	Anethole 1N, 2N, 3N, 5N, 6Y—III (Joint FAO/WHO Expert Committee on Food Additives, *F.A.O. Nutr. Mtg Rep. Ser.* 1967, **44A**, 7; WHO/Food Add./68.33)	50	

Abbreviation	Compound, track and reference	NEL (mg/kg)	Structure
BiP	Biphenyl 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33N—III (Ambrose *et al. Fd Res.* 1960, **25**, 328)	50	
TS	Tin salts (organic) 1N, 2N, 3Y, 4N—III (de Groot *et al. Fd Cosmet. Toxicol.* 1973, **11**, 19)	50	$Sn^{+2}(C_4H_4O_6)^{-2}$ $Sn^{+2}(C_2O_4)^{-2}$
ECH	Ethylene chlorohydrin 1N, 2N, 3Y, 4N—III (Oser *et al. Fd Cosmet. Toxicol.* 1975, **13**, 313)	45	$ClCH_2CH_2OH$
CHA	Cyclohexylamine hydrochloride 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19N, 23N, 24N, 25N, 26N, 22N, 33N—III (Gaunt *et al. Fd Cosmet. Toxicol.* 1976, **14**, 255)	30	
DNB	3,5-Dinitrobenzamide 1N, 2Y—III (Kerr *et al. Toxic. appl. Pharmac.* 1965, **7**, 488)	30	
YAB	Yellow AB (1-phenylazo-2-naphthylamine) 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33N—III (Hansen *et al. Toxic. appl. Pharmac.* 1963, **5**, 16)	25	
YOB	Yellow OB (1-*o*-tolylazo-2-naphthylamine) 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33N—III (Hansen *et al. Toxic. appl. Pharmac.* 1963, **5**, 16)	25	
Rd9	D & C Red No. 9 1N, 2N, 3Y, 4N—III (Davis & Fitzhugh, *Toxic. appl. Pharmac.* 1962, **4**, 200)	25	

Rd10	D & C Red No. 10 1N, 2N, 3Y, 4Y, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33N—III (Davis & Fitzhugh, *Toxic. appl. Pharmac.* 1963, **5**, 728)	25	
NAH	Isonicotinic acid hydrazide 1N, 2N, 3N, 5N, 6N, 7Y, 8N, 10N, 11Y, 33N—III (Harper & Worden, *Toxic. appl. Pharmac.* 1966, **8**, 325)	25	
MCB	2-Methoxy-3,5-dichlorobenzoic acid 1N, 2N, 3Y, 4N—III (Edson & Sanderson, *Fd Cosmet. Toxicol.* 1965, **3**, 299)	16	
PCF	3-Phenyl-2-carbethoxyfuran 1N, 2N, 3N, 5N, 6N, 7Y, 8N, 10N, 11N, 12Y, 13Y, 14Y, 15N, 33N—III (GRAS submission)	15	
SCP	Sodium 2,2-dichloropropionate 1N, 2N, 3Y, 4N—III (Weil & McCollister, *J. agric. Fd Chem.* 1963, **11**, 486)	15	
Ron	Ronnel 1N, 2N, 3Y, 4N—III (McCollister *et al. J. agric. Fd Chem.* 1959, **10**, 689)	15	
BCPM	Bis-(*p*-chlorophenoxy)methane 1N, 2N, 3Y, 4N—III (Weil & McCollister, *J. agric. Fd Chem.* 1963, **11**, 486)	15	
DMA	Dimethyl anthranilate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28N, 30Y, 31N, 32N, 22N—III (Gaunt *et al. Fd Cosmet. Toxicol.* 1970, **8**, 359)	15	
NNMC	α-Naphthyl *N*-methylcarbamate 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33N—III (Weil & McCollister, *J. agric. Fd Chem.* 1963, **11**, 486)	10	

Abbreviation	Compound, track and reference	NEL (mg/kg)	Structure
Ses	Sesone 1N, 2N, 3Y, 4N—III (Carpenter *et al. J. agric Fd Chem.* 1961, **9**, 382)	10	
NTU	α-Naphthylthiourea 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19N, 23Y, 27Y, 28Y, 29N, 33N—III (Fitzhugh, *Proc. Soc. exp. Biol. Med.* 1947, **64**, 305)	7·5	
Lin	Linuron 1N, 3N, 3Y, 4N—III (Hodge *et al. Fd Cosmet. Toxicol.* 1968, **6**, 171)	6	
Met	Methomyl 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20N, 22N, 33N—III (Kaplan & Sherman, *Toxic. appl. Pharmac.* 1977, **40**, 1)	5	
Ara	Aramite 1N, 2N, 3Y, 4N—III (Oser & Oser, *Toxic. appl. Pharmac.* 1962, **4**, 70)	5	
HCP	Hexachlorophene 1N, 2N, 3Y, 4N—III (Nakaue *et al. Toxic. appl. Pharmac.* 1973, **24**, 239)	3·7	
DNTa	3,5-Dinitro-*o*-toluamide 1N, 2Y—III (Weil & McCollister, *J. agric. Fd Chem.* 1963, **11**, 486)	3·1	
DCBN	2,6-Dichlorobenzonitrile 1N, 2N, 3Y, 4N—III (van Genderen & van Esch, *Fd Cosmet. Toxicol.* 1968, **6**, 261)	2·5	
Dod	Dodine 1N, 2N, 3N, 5N, 6N, 7N, 16N, 17N, 19Y, 20N, 22N, 33N—III (Levinskas *et al. Toxic. appl. Pharmac.* 1961, **3**, 127)	2·5	
DBTC	Di-*n*-butyltin dichloride 1N, 2N, 3Y, 4N—III (Gaunt *et al. Fd Cosmet. Toxicol.* 1968, **6**, 599)	2	

Rue	Ruelene 1N, 2N, 3Y, 4N—III (McCollister *et al. Fd Cosmet. Toxicol.* 1968, **6**, 185)	2	
Draz	Drazoxolon 1N, 2N, 3Y, 4N—III (Clark & McElligott, *Fd Cosmet. Toxicol.* 1969, **7**, 481)	1·5	
CPCB	*p*-Chlorophenyl-*p*-chlorobenzenesulphonate 1N, 2N, 3Y, 4N—III (McCollister *et al. Fd Cosmet. Toxicol.* 1964, **2**, 563)	1·25	
TDS	2,4,5,4′-Tetrachlorodiphenyl sulphide 1N, 2N, 3Y, 4N—III (Verschuuren *et al. Toxicology* 1973, **1**, 63)	0·5	
HClB	Hexachlorobenzene 1N, 3N, 3Y, 4N—III (Kuiper-Goodman *et al. Toxic. appl. Pharmac.* 1977, **40**, 529)	0·5	
Myl	Mylone 1N, 2N, 3N, 5N, 6N, 7Y, 8N, 10N, 11Y, 33N—III (Smyth *et al. Toxic. appl. Pharmac.* 1966, **9**, 521)	<0·5	
MMc	Methylmercuric chloride 1N, 2N, 3Y, 4N—III (Ikeda *et al. Toxicology* 1973, **1**, 361)	0·03	CH_3ClHg