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THE BIOLOGICAL BASIS AND MEASUREMENT OF THRESHOLDS

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"Was ist das nit gifft ist: alle ding sind gifft/vnd nichts ohn gifft/Allein die dosis macht das ein ding kein gift ist." (1)

"What is it that is not poison? All things are poisons and none that are not. Only the dose decides that a thing is not poisonous". (2)

INTRODUCTION

The above famous statement was made by Paracelsus in 1538 in reply to the criticism by academic medical men about his use of poisons to treat the sick. In his *Defensiones* he illuminates the fundamental issue that the distinction between poisonous and nonpoisonous substances is not real; the dose can move a substance from being nonpoisonous (and sometimes even beneficial) to poisonous. The thresholds for such and other actions depend on measurement. For example the auditory threshold, i.e. the least intensity at which a given sound can be perceived, is defined as an intensity of sound emitted. As knowledge of biological mechanisms increases, it may be possible to show that when a sound is emitted but not perceived, several steps in the complex process of hearing have been activated but the whole process not completed. In a similar way, as mechanisms of toxicity become established in molecular terms, possibilities emerge for answering the question of whether a threshold actually exists and explaining it in biological terms.

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Single molecules of a macromolecule (such as an enzyme or a receptor) are either active or inactive, although the activity or properties of the macromolecule are sometimes changed; e.g. acetylcholine on the nicotinic receptor (3), the regulation of enzymes by formation or breakdown of the phosphoenzyme (4), or the blocking of an enzyme catalytic center such as acetylcholinesterase by reaction with organophosphorus compounds (5, 6). Thus the control of metabolism and function is often entirely analogous at the molecular level to the perturbation of physiological systems by inhibitors and toxins. The occupancy of certain crucial sites in some parts of the macromolecule in vivo and whether this measurable change in activity becomes translated into a clinical or other response define the threshold question.

The above comments concern acute phenomena. When occupancy by a toxin of a catalytic site or receptor is maintained, the plasticity of biological systems is such that the primary response may become neutralized. Thus, if rats are exposed to an organophosphorus compound over a long period of time during which the acetylcholinesterase is inhibited in the brain, the initial rise in the concentration of acetylcholine is followed by a reduction to normal concentrations (7), i.e. the system adapts to the changed circumstances, presumably by producing less acetylcholine.

Toxicologists are mainly concerned with thresholds of chronic rather than acute exposure to chemicals. It is vital to know whether acute exposure leads to chronic toxicity or disease or whether chronic exposure will eventually lead to an accumulation of individual undetectable effects until clinical signs or syndromes appear. An empirical approach to such questions leads to the impossible philosophical conundrum of proving a negative. However, as knowledge of mechanisms increases one can ask more fundamental questions. For example, if after administration of a toxic chemical a degree of reaction with a relevant receptor or target can be measured, does this lead to disease?

For practical purposes many methods for the determination of threshold or no-effect levels depend on mathematical models of varying complexity. However, as noted by the European Chemical Industry Ecology and Toxicology Center, "the use of generalised mathematical schemes for deriving safety factors is to be depreciated because they cannot accommodate the wide variation in animal and human response and the variable quality and quantity of much of the data available. There is no alternative to the use of expert scientific judgement in this matter on a case by case basis" (8).

Invoking expert scientific judgment implies that an exact answer to the question cannot be given. The purpose of this paper is to emphasize the kind of biological information required for a logical approach to the problem. This includes measures of received dose rather than the concentration in the environment external to the animal or human, and knowledge of mechanisms derived from experimental studies on animals.

MECHANISMS OF TOXICITY

Although toxicity to a mammal is observed as clinical signs and symptoms of dysfunction, we can now subdivide the whole poisoning process into several well-defined aspects (9; Figure 1).

After entry of the chemical by several routes, the toxicity process is represented as four interconnecting compartments: They are in order from left to right: 1. all systems that influence the *delivery* of the chemical or a metabolically derived toxic derivative to its site(s) of action; 2. the interaction (called the *early reactions*) of the proximal toxin with targets (analogous often to pharmacological receptors) that are usually, but not exclusively, macromolecules; 3. the induction following the early (primary) reaction(s) of a cascade of *biochemical and physiological changes;* 4. the *consequences to the organism* that are seen as clinical signs, symptoms, or syndromes, which may range from serious impairments of function (as seen in many neuropathies) to minor skin conditions. This scheme can be extended ad infinitum with many details of toxic processes. In its present form, however, it concentrates the mind on four aspects of the toxic process that must often be studied using different experimental approaches.

The question of whether a threshold exists can now be refined and extended. Instead of asking whether a given dose leads to clinical signs of disease, one can ask whether a given degree of reaction with the target leads to clinical signs; whether a given reaction with the target causes biochemical, physiological, or

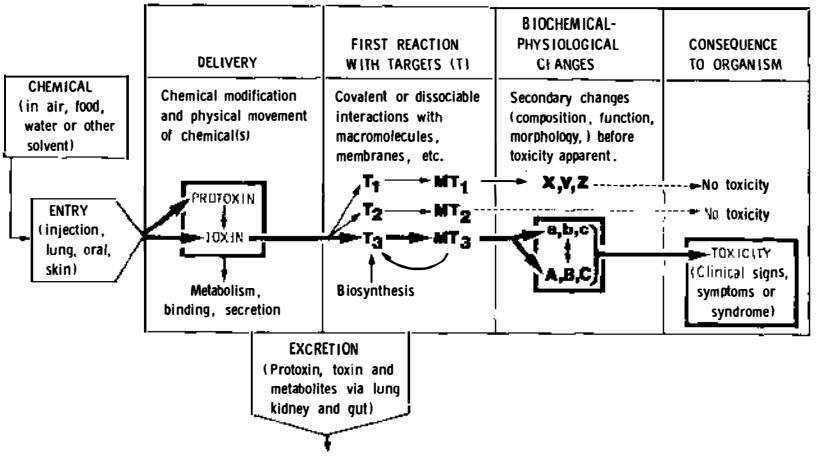


Figure 1 Scheme for developing toxicity due to chemicals. Heavy lines/letters = toxicity, light lines = less or no toxicity. T_1, T_2, T_3 = targets; MT_1, MT_2, MT_3 = modified targets. A, B, C, X, Y, Z, a, b, c = independent or linked changes. Acute or chronic toxicity differ by the time in one or more phases. Adapted from (9).

morphological changes; and what the relationships are between such changes and the clinical signs.

The identification of the relevant target is a vital component of such an approach and is a rate-limiting step in the progress of research. The development of sensitive analytical methods and the ability to produce monoclonal antibodies by the new techniques of molecular biology have, in principle, solved most problems of measuring how extensively chemicals react with their targets. Comparison of the properties of such proteins, (produced in quantity by bacteria separated by cloning techniques) with the same protein present in vivo, and perhaps attached to membrane, will aid in this process. Comparison of the properties of the target from the experimental animal with properties of the target from man (using postmortem material) is also possible. In the experimental animal it should prove increasingly possible to relate the degree of reaction of the chemical with the target (which is a measure of tissue or cell dose) to subsequent biological events.

EXPOSURE AND MEASUREMENT OF IN VIVO DOSE OF TOXIC CHEMICAL

Research on animals to produce information about the effects on man of exposure to chemicals is based on the premise that laboratory animals' component parts (enzymes, macromolecules, membranes, etc) are similar to man's, but that major differences lie in the relationships among the parts and their quantitative and kinetic properties. Major interspecies differences in sensitivity to a chemical are often due to great differences in the processes shown in the delivery section of Figure 1. Rapid metabolism and/or excretion of the toxin are often major factors. As shown in Figure 1, excretion may result from the early reactions phase. Covalent interaction results in the breakdown of the toxic chemical. Thus if the interaction does not result in toxicity (cf Figure 1), the process may be regarded as detoxification. Such a process has been experimentally demonstrated in the rat for the reaction of carboxylesterase and the highly toxic organophosphorus compound, Soman (10). Binding of chemicals to plasma proteins has been much studied and provides a depot of the chemicals and also increases the dose necessary to cause toxicity by reducing the circulating concentration of compound. An example of this is the influence on toxicity of the binding of trimethyltin to rat hemoglobin but not to the hemoglobin from other species [(11); see conclusions section].

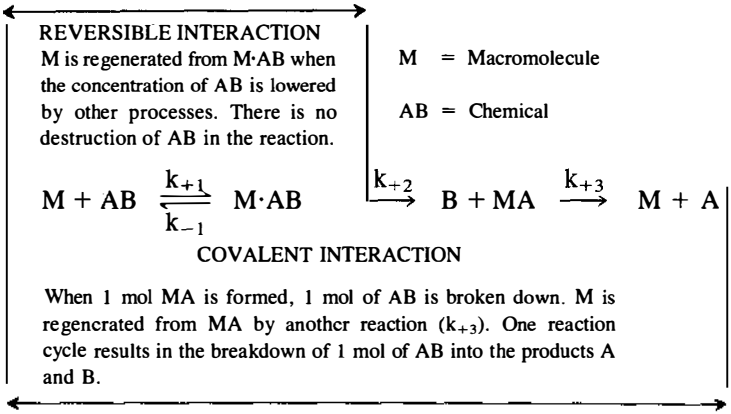
An accurate measure of absorbed dose is a requirement for using the results of laboratory animal studies to predict quantitative effects in man. As a measure of received dose of organophosphorus compounds, the activities of acetylcholinesterase in erythrocytes and NTE in leucocytes are useful in acute and chronic poisoning.

One of the most helpful developments is the use of hemoglobin as a protein containing amino acid residues with a reactivity for many substances: Hemoglobin also has a long life in man (120 days). Thus exposure can be estimated some time after the event. The theoretical basis for its use has been well discussed (12–18), and the methodology for the measurement of specific adducts in hemoglobin has been worked out (12, 19, 20). The principle of the method has much potential for monitoring received or absorbed doses resulting from exposure of humans and laboratory animals. Problems remain to be solved and the interpretation of results is difficult for electrophiles of a wide range of reactivity produced in vivo by metabolic activation from inactive compounds. The use of the information for risk assessment also continues to provoke much discussion. Other possibilities are the use of other proteins to directly measure tissue-dose in experimental animals and the use of a very recent development employing a modified and more sensitive Edman degradation method that does not require hydrolysis of the hemoglobin. This latter method also has some potential, where the nature of the exposure is unknown, to identify the resultant adducts (21).

Although the main thrust of these developments concerns genotoxic agents, methods could be developed for other chemicals causing other types of toxicity.

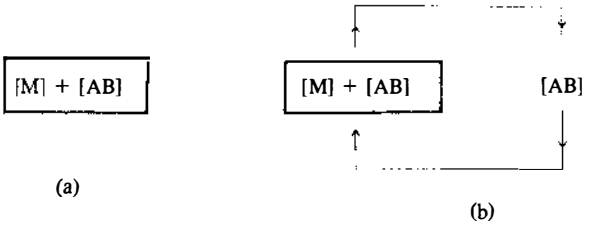
TYPES OF REACTION OF CHEMICALS WITH MACROMOLECULES

Reactions with targets such as those shown in the early reactions section of Figure 1 are of two types, reversible and covalent.



The reaction mechanisms have consequences for the toxic process. It is probable that for some reactions the time during which the target is modified is an important factor in the development of toxicity. For example the development of morphological change in the cerebellum after methylmercury poison-

ing in rats seems to depend not only on the concentration of the methylmercury in the brain but also on the length of time it is there (22). Development of signs of poisoning may also depend on the speed at which the target is modified (23, 24).



Affinity constants or reaction rates are almost always measured in vitro with the macromolecule (enzyme or receptor) and the ligand or reactive chemical in a small volume (cf (a) above). Under these circumstances the molar concentration of the macromolecular component can approach that of the chemical reactant, i.e. [AB]≅[M] and AB (mol)≅M (mol). The relationship between the degree of reaction and concentration of reactant [AB] will have a different slope from that when [AB]>>[M]. In vivo the macromolecule is often perfused with a large volume containing [AB] and under these circumstances the determining feature is that the quantity of AB (mol) >> M (mol). Even for extremely toxic substances this condition applies. For example the amount of acetylcholinesterase in rat brain is approximately 100 pmol (calculated from an acetylcholinesterase activity in rat brain of 30 μmol/min/brain and a catalytic center activity of 295,000/min). The toxicity of the nerve gas Soman to rats is 80 μg/kg when given subcutaneously (10); this is 88 nmol/200g rat, an excess approaching 10^3 over the 100 pmol amount of acetylcholinesterase.

It seems probable that AB (mol) >> M (mol) will be the normal condition, and the degree of reaction in vivo will be linearly related to the dose administered. Reaction of metabolites of trans-4-dimethylaminostilbine in rats with the protein and nucleic acids of a variety of tissues, as well as hemoglobin, have been shown to relate linearly to doses over a range of 5×10^{-10}–3.5×10^{-5} mol/kg (15, 25, 26).

EXAMPLES OF VARIOUS NEUROTOXIC CHEMICALS

In the following pages I use examples of chemicals that cause neurotoxicity to demonstrate how knowledge of mechanisms of toxicity influences views about thresholds (a dose just below that producing a response). These thresholds concern not only clinical signs but also other obligatory steps following from the early and primary reaction of the chemical with the target (cf Figure 1).

Wherever possible, I discuss methods to measure in vivo dose and how the results of experimental studies can be linked to observations in man.

Organophosphorus Compounds (Acute Toxicity)

It has long been known that the acetylcholinesterase of erythrocytes is similar to that of brain, and exposure may be monitored by its measurement (27). The threshold for untoward effects has been discovered empirically in man by routine measurements performed during the medical supervision of the spraymen engaged in World Health Organization spraying programs to control vector-borne disease. Since most organophosphorus compounds are uncharged and lipid soluble they penetrate all tissues including the brain. It is known that dimethylphosphorylated acetylcholinesterase is relatively unstable; it spontaneously reactivates to yield the parent active enzyme and, at a slower rate, changes to a stable monomethylphosphorylated enzyme known as aged enzyme (28). After a day of spraying, these reactions occur, and the activity of acetylcholinesterase the next day is a measurement of aged enzyme (29). If the dose absorbed each day is repeated then the aged enzyme also increases from day to day. Because both the mechanisms of reaction of organophosphorus compounds with acetylcholinesterase and the properties of the phosphorylated enzyme are known, the spraying operation can be conducted safely. Such knowledge and monitoring procedures have allowed large numbers of patients infected with *Schistosoma hematobium* to be treated completely safely by the oral administration of metrifonate (O,O-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate) (29). Few or no untoward effects were seen (30, 31).

Thus the target involved in the acute toxicity of organophosphorus compounds is known, a similar target is present in an accessible tissue (erythrocytes), reaction of the target with the chemical (inhibition of enzyme activity) can be measured, and in animals this may be related to clinical signs or acetylcholine concentrations in nerve tissue. This allows the threshold to be defined. The properties of the target may be compared between animal and man and the threshold established in man.

The dose of organophosphorus compound that produces symptoms may of course be different in various species. Metrifonate is not an inhibitor of acetylcholinesterase but breaks down spontaneously to dichlorvos, a very active inhibitor. In Table 1 it is shown that 15–20 times as much metrifonate is required in the hamster as is required to produce the same inhibition in man. This difference is probably due to a more rapid disposal of the active inhibitor dichlorvos in the hamster (Nordgren, I., unpublished, 1985) than in man (32). Thus although the threshold for clinical effect, expressed in terms of percentage inhibition of acetylcholinesterase, is the same in hamsters and man, the dose

Table 1 Inhibition of erythrocyte cholinesterase in hamsters and man after treatment with metrifonate.

Metrifonate oral dose (mg/kg)	Acetylcholinesterase (% of control)	
	Human[a]	Hamster[b]
7.5	80	—
10	50	—
12.5	65	—
7.5–60	—	100
120	—	94
150	—	68
200	—	47

[a]Taken from (21)

[b]Unpublished results W. N. Aldridge & B. W. Street 1984. Biomolecular rate constants for inhibition of acetylcholinesterases by dichlorvos are for hamsters ($1.26 \times 10^5 M^{-1}min^{-1}$) and for human $1.17 \times 10^5 M^{-1}min^{-1}$ (28)

administered to achieve this inhibition is 15–20 times as high in hamster as in man.

Organophosphorus Compounds (Chronic Delayed Neuropathy)

Some organophosphorus compounds produce a condition known as delayed neuropathy. Signs of this axonal neuropathy take 10–30 days to appear in animals and man; the early primary reaction is with a membrane-bound neuropathy target esterase (NTE) present in the peripheral and central nervous tissue (33–35). The structure-activity relationships for a large number of organophosphorus compounds are known, and all take part in the reaction scheme shown in Figure 2. For the development of delayed neuropathy, 70–80% of NTE must be phosphorylated or phosphonylated followed by the removal of one of the groups attached to phosphorus (aging reaction). If the aging reaction does not occur, even though the esterase activity of NTE is inhibited, delayed neuropathy does not develop. The aging reaction cannot on chemical grounds occur when NTE is phosphinylated (both R^1 and R^2 directly linked to phosphorus), carbamylated, or sulfonylated (36) (cf Figure 2). Animals pretreated with the above three classes of compounds in doses sufficient to inhibit NTE by 70% do not develop delayed neuropathy. If an organophosphorus compound able to cause delayed neuropathy is administered to such pretreated animals, the animals are protected and no disease occurs.

When NTE is phosphinylated or sulfonylated, hen esterase activity slowly reappears with a half-life of 4.5–5.0 days (Figure 3), presumably due to resynthesis of new enzyme. When a hen is dosed with phenylbenzylcarbamate, the carbamylated NTE is unstable and reactivates (cf. Types of Reaction of

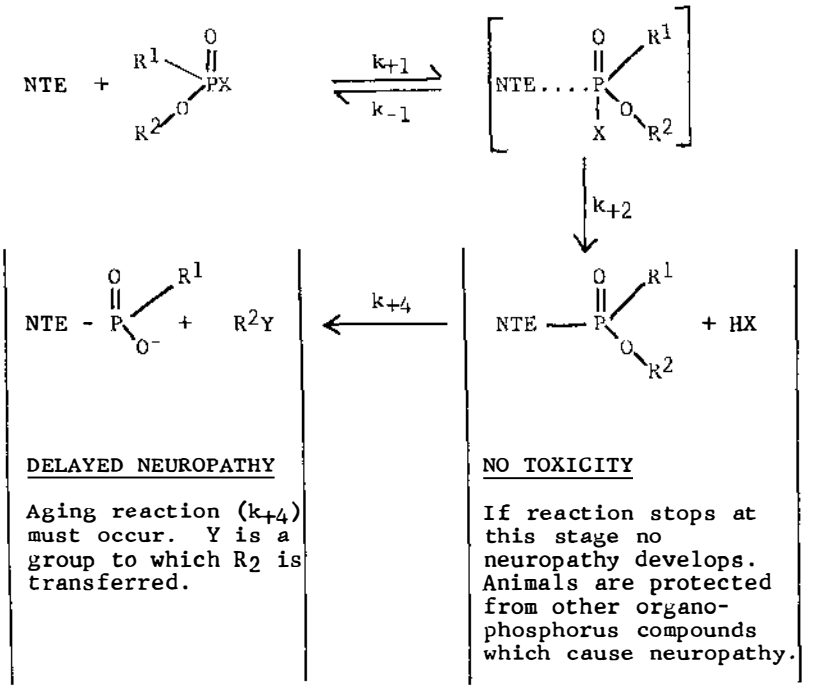


Figure 2 Reactions necessary for initiation of delayed neuropathy by organophosphorus compounds. R^1 maybc linked to P cither directly or via O, S, or N. R^2 must be linked to P by O, S, or N. Chemical evidence for aging has only been provided for transfer of R^2 of R^2O to $Y(k_{+4})$ and not for R^2S- or R^2NH- (43).

Chemicals with Macromolecules) with a half-life of approximately 6 hr (Figure 3). Hens pretreated with any of the compounds shown in Figure 3 are protected until NTE inhibition is reduced to 28–35%—i.e. the animals are protected until approximately 70% of the catalytic centers are unoccupied and able to be phosphorylated followed by aging after treatment with a neuropathic organophosphorus compound (36, 37).

These observations prove that NTE is the primary target involved in the reaction with certain neuropathic organophosphorus compounds. Biochemical/physiological changes following the primary reaction and the appearance of clinical signs of neuropathy (at 10–14 days in the hen and 14–30 days in man) have not been defined, and the nature of the biological cascade in this "silent" period is unknown. The threshold of 70–80% inhibition or phosphorylation of NTE in hens after an acute dose is now well established (38).

Three questions may be asked: (*a*) How can information obtained in the hen be applied to man? (*b*) What is the threshold with chronic doses? (*c*) How can humans be monitored for toxicologically significant exposure?

Having established the properties of NTE in the hens, we can examine the

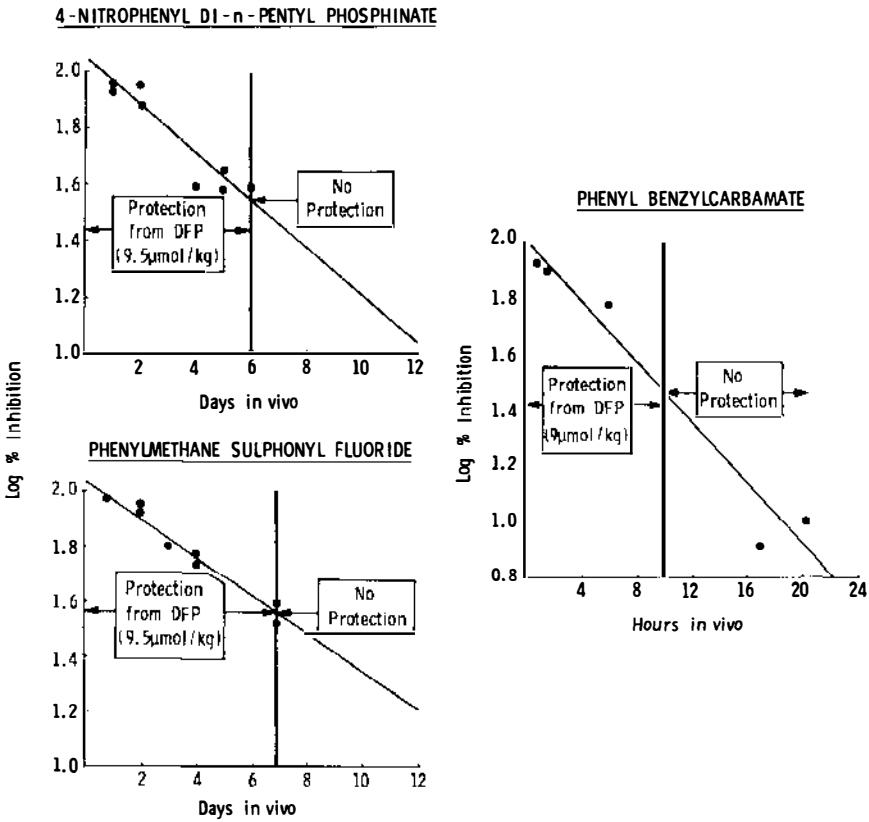


Figure 3 Protection of hens from delayed neuropathy by pretreatment with various compounds. DFP is diisopropylphosphorofluoridate. Inhibition of neuropathy target esterase (NTE) is plotted as percentage of the maximum inhibition measured. Data from (36, 37).

enzyme from human tissue. Except for minor differences, hen and human NTE are similar (39, 40). Since there may be major differences in the rates and routes of metabolic disposal of organophosphorus compounds in hens and humans it is not possible to predict from in vitro inhibition studies of NTE the effective dose in the two species. Comparison of the relative inhibitory power of the proximal inhibitory organophosphorus compounds against those of acetylcholinesterase and NTE in microsomes from hen and human brain give a good indication of human susceptibility to this chronic toxicity relative to acute toxicity (40, 41). Measurement of acetylcholinesterase and NTE after dosing a few hens also indicates the potential or lack of potential for delayed neuropathy of the compound. After such experiments have been done it will be possible to predict whether humans suffering from acute poisoning will develop delayed neuropathy later.

Much of the experimental work on delayed neuropathy has been done with single or at most a few doses of organophosphorus compounds. Does the threshold of 70% inhibition of NTE apply for chronic administration, or is there an accumulation of morphological changes with lower inhibition? Hens were maintained on a daily dose of mono-2-methylphenyl diphenyl phosphate such that a steady-state 50% inhibition of NTE was obtained for 10 weeks (42). No neuropathy was found. Raising the dose, after 50% inhibition had been maintained for several weeks, to produce 70–90% inhibition resulted in neuropathy 10 days later (42). These experiments show that an acute dose of approximately 50 mg/kg produces neuropathy whereas a total of 175 mg/kg given over 10 weeks in daily doses does not; measurement of the inhibition of NTE shows that the toxic chemical was delivered to the target. Thus, for hens, it appears that the threshold is approximately the same in terms of degree of reaction with NTE for both acute and chronic doses.

NTE, defined by its sensitivity to organophosphorus compounds, is a B-esterase. It seems, however, to be a unique B-esterase; when it ages the released group is attached to a neighboring group (43). This transferred group has been characterized by its volatility after treatment with alkali (43, 44). Other phosphorylated esterases when they age release the group into the medium. Using this unique characteristic NTE has been identified in tissues other than those of the central and peripheral (45) nervous system and particularly in spleen, placenta, thymus, and circulating leucocytes (35, 46–50). The determination of NTE activity in leucocytes in hens (42) and humans (51, 52) is related to the previous dose of active inhibitor and takes into account the bioactivation of inactive precursors and detoxification. Although the method requires more validation, it could provide a valid monitoring procedure in cases of occupational, accidental, or intentional exposure.

Acrylamide (Peripheral Neuropathy)

Acrylamide causes a peripheral neuropathy in many species of animal (53), including man (54). Acrylamide in rats is an uncharged and highly water soluble compound that mixes uniformly with the body water. It reacts with cysteine residues in hemoglobin to produce stable adducts (55), and a method has recently been developed for their measurement (56). The macromolecular target in the early primary reaction that leads to peripheral neuropathy is unknown. It is uncertain whether the proximal toxin is acrylamide or metabolite (57). Nevertheless in an occupational exposure it should be possible to examine those exposed for clinical signs, to determine cysteine adducts in hemoglobin and to calculate the integrated dose of acrylamide absorbed. A threshold for clinical peripheral neuropathy may thus be established in both man and other animals. Similar studies to define a threshold dose in relation to morphological findings (58, 59) would enable us to establish, in animals, the relationship

between dose received and discrete morphological findings or clinical signs of toxicity.

2,5-Hexanedione (Hydrocarbon Neuropathy)

It has now been established that the predominantly peripheral neuropathy caused in rats by hexane, 2-hexanol, 2-hexanone (methyl n-butyl ketone), and 2,5-hexanediol result from metabolism in vivo to a common proximal toxin, 2,5-hexanedione (60, 61; cf Figure 4). Methyl n-butyl ketone causes a similar neuropathy in man (62, 63) and it is metabolized to 2,5-hexanedione. The morphological characteristics have been well described (64, 65) and it has been shown that 2,5-hexanedione, acrylamide, and organophosphorus compounds produce different patterns of morphological change (66). The structural deter-

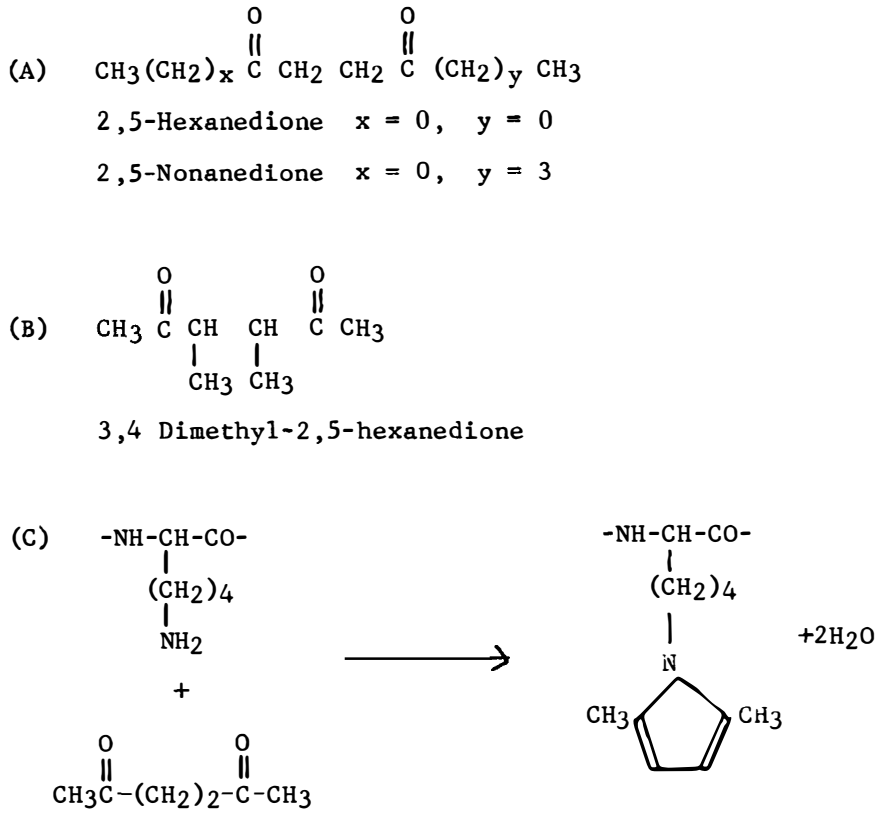


Figure 4 Neuropathy caused by 1,4-diketones. (A) Structural requirements in diketones; (B) 3,4-dimethyl-2,5-hexanedione, which reacts with primary amines faster than 2,5-hexanedione, and is 20–30 times more neuropathic (73); (C) postulated reaction of 2,5-hexanedione with amino group in lysine to form a 2,5-dimethylpyrrole derivative (72, 74).

minant within 2,5-hexanedione essential for the production of neuropathy is two oxygen atoms (of the keto groups) separated by four carbons (Figure 4). Other compounds with longer carbon chains that contain this grouping or can be metabolized to such a structure are also neuropathic (67–71). Thus, although the complete structural requirements for neuropathic potential have not been worked out, e.g. whether x in Figure 4 can be more than 0, the requirement for the 1,4-diketone grouping seems absolute.

The requirement for a 1,4-diketone structure has been utilized to formulate a chemical hypothesis that involves reaction with primary amino groups (presumably in proteins) to form a stable 6-membered pyrrole ring adduct (72). Other diketones with the keto groups in the 1,3-, 1,2-, or 1,5- positions are not neuropathic (68). The hypothesis has also received support from the observation that a 3,4-dimethyl substitution in 2,5-hexanedione increases reactivity with amino groups to form a pyrrole and also decreases the dose required to produce a neuropathy like that produced by 2,5-hexanedione (73, 74). Precisely which macromolecules react with 2,5-hexanedione and initiate the toxicity is not known, but they may well be neurofilaments, which accumulate at the nodes of Ranvier as an early event in the developing neuropathy (64). It is possible that after the primary reaction with amino groups in the neurofilament protein, the pyrrole adduct auto-oxidizes to form a cross-linking reactive center (74).

Comparative dose-response relationships have been worked out for 2,5-hexanedione and several compounds metabolized to it (Table 2). Even though the circulating concentrations of 2,5-hexanedione differ by a factor of 8, the doses required to produce neuropathy are remarkably similar (*last column,* Table 2). This is surprising for a compound with a disposal half-life in plasma of approximately 6 hr (60). If reaction with neurofilaments is a primary event an explanation may be available. Passage of neurofilaments down the axon is part

Table 2 Relative neurotoxicity of various compounds in relation to their conversion to 2,5-hexanedione[a]

Compound	Time to clinical end point (days)	No. of doses	2,5-Hexanedione: Peak serum concentration (μmol/ml;B)	Dose (A × B)
2,5-Hexanedione	17	13	4.59±0.79	59.7±10.3
5-Hydroxy-2-hexanone	22	16	2.56±0.41	41.0± 6.6
2,5-Hexanediol	29	21	1.92±0.47	40.3± 9.9
Methyl n-butyl ketone	56	40	0.89±0.10	35.6± 4.0
2-Hexanol	83	61	0.60±0.05	36.6± 3.0

[a]Data from (60). Rats were dosed orally with 6.6 mmol of each compound per kg for 5 days a week.

of the slow component of axonal transport that moves down the nerve at 1–2 mm per day (75, 76). Passage down some long nerves may take up to 50 days and during repeated exposure 2,5-hexanedione would have opportunity to react with neurofilaments over a long period; this could be the explanation for its apparent cumulative behavior. Thus the threshold is likely to be low—certainly much less than 0.6 μmol/ml—and is determined by the life of a particular organelle. Factors such as repair on its way down the nerve or resynthesis of new protein will affect this threshold.

2,5-Hexanedione has also been shown to react with proteins in the erythrocyte (presumably mainly hemoglobin, the most abundant protein), and the pyrrole adducts can be determined (74, 77). No experimental work has been published on the stability of these pyrrole adducts, i.e. whether they are like alkylated adducts and persist for the life of the erythrocyte. Further experimental work and examination of those exposed should allow determination of the dose-response relationships and the threshold dose in experimental animals and man. The long life of hemoglobin and its disposal when it becomes "old", rather than the first-order turnover process seen for most proteins, probably show many kinetic similarities to the slow passage of neurofilaments down a nerve axon with their disposal at the end (78, 79). Both of the above processes have a kinetic analogue in the relationship between methylmercury in blood and the progression of the methylmercury down the hair as it grows (79a).

Carcinogenic and Mutagenic Chemicals

Carcinogenesis is not considered in detail here because it has often been reviewed (80). As new information is obtained on the mechanisms involved in the multistaged process leading from initiation to tumor (e.g. information on promotion, oncogenes, etc), it may be possible to consider thresholds for weak carcinogens. This is in contrast with a complete and strong carcinogen, such as diethylnitrosamine, which shows the characteristic of extending the time required for detection of liver tumors as its dose is reduced. In other words the fundamental absence of a threshold is indicated (81).

On general grounds it seems probable that some as yet incompletely defined modification by chemicals of the DNA template in a cell, leading to nonlethal mutation, is a process that has no threshold. Initiation requiring chemical modification in the DNA in principle could also be brought about by changes in the repair-synthesis system where a one-to-one molecular relationship between DNA and enzyme occurs; for example, the influence of beryllium on the fidelity of DNA synthesis (82). The results of long-term animal experiments, indicating chemically induced increased incidence of tumors in the progeny after pre- or postnatal exposure of rats to nitrosoureas, urethane, or Xrays, should provoke much thought and attention (83–87).

CONCLUSIONS

The intent of this paper is to consider the existence of threshold from a fundamental biological standpoint and not to concentrate on the practical problems of those who have to set limits of exposure for populations. In this context attempts to decide, in a particular case, whether in principle a threshold exists, are separate from the problem of devising sound methods to test whether it does.

When a biological function that depends on the collaborative action of a large number of the same macromolecules such as receptors or enzymes is deranged then it seems probable that a threshold exists. The action of a number of molecules of a receptor or enzyme could be prevented or modified without any demonstrable change in biological function. However, when biological function is dependent on the action of one molecule such as DNA or those molecules (enzymes) associated with it in a one-to-one molecular relationship, then the existence of a threshold seems fundamentally unlikely. Acceptance of this view, of course, means that exposure to most toxic chemicals at low concentrations for a lifetime will be harmless. In contrast, for those chemicals causing genetic changes this seems unlikely. Such statements ignore questions of risk involving the slope of dose-response curves (which need not be constant over the whole dose range) or the differing susceptibility of individuals. Practical decisions have to be made even though the risk is not zero.

As knowledge of mechanisms of toxicity of chemicals is extended it will become clear that different facets of the toxic process have different thresholds. For example when inhibition of an enzyme results in defined symptoms the thresholds for these two measurable parameters will be different. In general if we move from right to left in Figure 1, from the clinical signs towards measurement of circulating toxin, several thresholds occur at lower concentrations of toxin. These dose-response relationships can be worked out in laboratory animals and will provide the scientific background for the interpretation of the often limited data (dose and clinical signs) available for man.

An illustration of this principle is shown by the action of trimethyltin which causes neuronal necrosis in rats only in the hippocampus, amygdaloid, or pyriform cortex (88, 89). The dose required to cause these effects is higher in rats than in such other species as the marmoset, hamster, and gerbil (11). This is undoubtedly due to the binding of trimethyltin to rat hemoglobin only, so that 70% of the administered dose is sequestered in an inactive form. The concentrations of trimethyltin in brain associated with neuronal necrosis in the above four species are similar (11) and there is no evidence of selective accumulation in the affected areas (89). It is thus possible to predict the dose that will be required to produce such lesions in man (11). Neuronal necrosis in

rats and other species of experimental animals is preceded by aggression (88), and this has been seen in some of the reported cases of poisoning in man (90–92). Even with considerable neuronal necrosis in rats, after a recovery period it is difficult to detect behavioral abnormalities (88), but deficiencies in memory and problem solving have been found (93, 94). Poisoned men who showed severe symptoms have subsequently been pronounced clinically normal (90–92). In this instance detailed information about the dose-response curve, threshold for neuronal necrosis, and early behavioral changes would provide a sound basis for predicting (a) what concentrations will not cause permanent loss of neurons in man, and (b) the relationship of these levels to initial symptoms. When the biochemical basis for its action is known another dose-response and threshold may be measured.

In these and similar studies the measurement of received or absorbed dose is vital. The increasing sophistication of detection, identification, and quantitation techniques now available allows such measurements. For covalently reactive chemicals the measurement of hemoglobin adducts has proved valuable and can be extended.

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