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A Critical Appraisal of the Threshold of Toxicity Model for Non-Carcinogens

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Abstract

Most regulatory agencies assume that there is no safe level of exposure to carcinogens but that a threshold, or "safe" exposure level exists for non-carcinogens. However, recent discoveries have cast serious doubt on the validity of this concept. Five examples of non-carcinogens without an apparent threshold (neonicotinoids, dioxin, dieldrin, endocrine disruptors, and sulfhydryl-reactive metals) are presented. It is also clear by now that the threshold model for non-carcinogens may seriously underestimate actual risk. Risk assessments can no longer assume thresholds for non-carcinogens when the shape of the dose-response curve is linear at low concentrations. Risk management of such chemicals should be based on the ALARA principle ("as low as reasonably achievable").

Keywords: Risk Assessment; Threshold; Carcinogen; ALARA

Introduction

There is no universally accepted dose response model for risk assessment of low level exposures to potentially toxic substances, mainly because there is no consensus on presence or absence of a threshold below which no adverse effects can be assumed to occur. The issue is readily apparent in carcinogenic risk assessment. The dose-response model for carcinogenic risk assessment depends on absence or presence of genotoxic potential [1]. Non-genotoxic carcinogens (hormones, tumor promoters, TCDD as examples) are assumed to be characterized by a "conventional" dose–response relationship which allows derivation of a no-observed-adverse-effect-level (NOAEL), and an insertion of an uncertainty (or safety) factor permits the derivation of permissible exposure levels at which no relevant human cancer risks are anticipated [2]. A far more stringent dose-response model was widely adopted for genotoxic carcinogens.

The Genetics Panel of the U.S. National Academy of Sciences' Committee on Biological Effects of Atomic Radiation (BEAR) recommended the linear non-threshold (LNT) dose–response model in 1956, abandoning the threshold dose–response for genetic risk assessments [3]. This recommendation was adopted by the Atomic Energy Commission for estimates of the cancer risk from radioactive fallout [4]. The point of departure is the assertion that the dose-response relationship for radiation-induced mutations is linear [5]. The NAS BEAR Committee Genetics Panel recommendation was quickly generalized to include somatic cells for cancer risk assessment and later was instrumental in the adoption of linearity for carcinogen risk assessment by the U. S. Environmental Protection Agency [6,7]. It was assumed that if "one hit" could cause a mutation and eventually result in cancer, then any exposure level could be associated with a finite cancer probability. The cancer risk assessment procedures adopted by the European Union scientific committee on occupational exposure limits (SCOEL) reserve the LNT dose-response model only for DNA reactive, tumour initiating genotoxic carcinogens, e.g., alkylating chemicals (vinyl chloride, 4-aminobiphenyl, diethylnitrosamine, acetaminofluorene, aflatoxin B_1) or ionizing radiation [8]. SCOEL does, however, also recognize genotoxic carcinogens with a practical threshold (formaldehyde, vinyl acetate), and health-based exposure limits may then be based on an established NOAEL [8]. The LNT dose-response model has also been challenged by several authors who hypothesized potential thresholds and protective mechanisms throughout the process from initial DNA damage induction to tumor formation [9-13]. A

sequential order of genome protection during carcinogenesis where genotoxicant scavenging, cellular efflux, DNA repair, elimination of damaged cells by apoptosis, autophagy, silencing by DNA damage-triggered replicative senescence, and finally, elimination of transformed (premalignant) cells by the immune system are thought to be responsible for a threshold in tumor formation. Within this context, the principle of Threshold of Toxicological Concern (TTC) has been developed by Kroes et al. [14]. The TTC approach applies a generic threshold for structural alerts of 0.15 μg/person/day (0.0025 μg/kg bw/day) but excludes high-potency genotoxic substances, such as aflatoxin-like compounds, N-nitroso-compounds, and azoxy-compounds, from consideration [15]. The TTC concept has been used by the US Food and Drug Administration (FDA) to establish "thresholds of regulation" for indirect food additives as well as by the Joint FAO/WHO Expert Committee on Food Additives for flavoring substances. TTC has also been proposed for assessment of prenatal developmental toxicity [16], and for safety evaluation of cosmetic ingredients, pharmaceutical manufacturing operations [17,18] and even for deriving target values for drinking water contaminants [19].

The threshold debate was compounded ever further by the observation that the neurotoxicity of non-genotoxic chemicals such as neonicotinoid insecticides and organic mercury may show dose-response relationships identical to that of an alkylating N-nitroso carcinogen such as diethylnitrosamine [20-23]. The common denominator of the dose-response relationship is irreversibility of receptor binding and irreversibility of the associated effect [21]. In fact, the discovery of the carcinogenicity of dimethylnitrosamine [24] which alkylates nucleic acids following enzymic hydroxylation [25], triggered the pharmacologist and cancer researcher Hermann Druckrey to put this concept to the test. Druckrey and Küpfmuller had hypothesized

many years earlier with theoretical approaches to dose-response relationships that irreversible receptor binding with an associated irreversible effect would lead to reinforcement of the effect by exposure time [26] (Table 1).

Druckrey and his associates successfully validated the Druckrey-Küpfmüller theorem in rat studies with diethylnitrosamine [27] and numerous other nitrosamines [28], as reflected in what is now known as the Druckrey-Küpfmüller equation:

$$dt^n = constant \quad (1)$$

where d=daily dose and t=exposure time to effect (liver cancer), and n=2.3 (diethylnitrosamine)

There is no valid reason to assume a threshold for non-carcinogens when their dose – response relationships are identical to that of diethylnitrosamine and their mechanisms of action suggest irreversible receptor binding associated with irreversible effects. Moreover, there is increasing evidence to suggest that the threshold model may seriously underestimate actual risks. Five examples of non-carcinogens that may not have a threshold are discussed.

Neonicotinoids

Sanchez-Bayo [29] and Tennekes [20] recently demonstrated that equation (1) also describes the (neuro)toxicity of neonicotinoid insecticides in arthropods. Moreover, the mechanism of action also shows similarities with that of alkylating nitrosamines. The neonicotinoid insecticides block nicotinic acetylcholine (nACh) receptors in the central nervous system of insects [30] which leads to irreversible neuronal damage [31]. The affinity of the neonicotinoid imidacloprid for the nACh receptor in insects is very high, and, unlike the normal neurotransmitter acetylcholine, acetylcholinesterase can not remove imidacloprid from the nACh receptor, although this concept has recently been disputed [32] in spite of previous assertions [30,31] by Bayer experts of irreversible receptor binding. Dissociation, if it occurs at all, is bound to be very slow, and cumulative nACh receptor binding leading to irreversible neuronal toxicity can be easily envisaged [33].

Due to their environmental properties, neonicotinoids are threatening the survival of invertebrates. Several persistent neonicotinoids (imidacloprid, clothianidin, thiamethoxam) with time-cumulative toxicity to arthropods are prone to leach from soils [34], and have been demonstrated to contaminate surface water in Europe and North America [34,35]. In the Netherlands, surface water contamination with imidacloprid has been demonstrated to correlate with decline of macro-invertebrates [36] and insectivorous birds [34,37], and entomological surveys in Dutch and German nature reserves have revealed a staggering decline of ground beetles and flying insects since the introduction of this ubiquitous pesticide in agriculture in the early 1990s [34,38]. The risks of imidacloprid's time-cumulative toxicity to non-target insects have clearly been underestimated, and a revision of risk assessment is imperative. The Druckrey-Küpfmüller equation (1)

with n ≥ 1 can serve as a dose-response model for risk assessment of compounds with time-cumulative toxicity. Carlborg pointed out that this equation is implied by a Weibull model for dose-response functions in carcinogenesis [39].

Dioxin

Lucier et al. [40] evaluated the effects of dioxins and pointed out that most, if not all, of dioxin's effects require interaction with a cellular protein, the Ah receptor. It is generally accepted that Ah receptor occupancy is linearly related to low cellular concentrations of dioxin [40]. Dioxin, like other Ah receptor agonists, induces an isoform of UDP-glucuronosyltransferase (UGT-1) by an Ah receptor-dependent mechanism [41]. This enzyme conjugates thyroxine (3,5,3',5'-tetraiodothyronine, T4), leading to its clearance. Metabolism of T4 and its consequent depletion from the blood relieves inhibition of TSH release from the pituitary by circulating T4 and causes the serum TSH concentration to rise, which is generally believed to promote the induction of thyroid tumors in rats and mice [41]. The low-dose linear responses of TSH and UGT-1 suggest the absence of a threshold for dioxin's effects on the thyroid [41].

Dieldrin

Similarly, the dose-response for promotion of liver tumor induction in mice by the non-genotoxic insecticide dieldrin was shown to be consistent with the absence of a threshold [42]. The accelerated velocity of liver tumor formation was shown to be linearly related to dieldrin dose.

Endocrine Disruptors

The absence of a threshold for endocrine disruption has also been demonstrated in an experiment concerning the regulation by estrogen of sex determination in reptiles [43]. Since endogenous estrogen is already above threshold for estrogen-mediated responses there can thus be no threshold for responses to exogenous chemicals that act as hormone mimics via estrogen receptor mechanisms [44].

Sulfhydryl Reactive Metals

In the case of organic mercury [23], the actual toxicant in the central nervous systems (CNS) is thought to be the divalent mercuric ion (Hg^{2+}) which is formed when organic mercury compounds such as methyl- and ethylmercury dealkylate. Once organic mercury compounds reach the brain tissue and dealkylate, Hg^{2+} gets trapped in the neurons, as it cannot permeate the blood-brain barrier. Hg^{2+} has electron-sharing facilities that can result in formation of covalent attachment to sulfhydryl groups of proteins, and binding of mercury species to thiol groups in amino acids, intracellular enzymes and structural proteins. It can be envisaged that mercury neurotoxicity could result from irreversible binding of mercuric ion to sulfhydryl groups of organic macromolecules [23].

Likewise, subtle effects on children's health of other sulfhydryl-reactive metals, such as cadmium (Cd), lead (Pb), and arsenic (As),

Reversibility of receptor binding	Receptor binding in relation to compound concentration	Reversibility of the effect	Effect in relation to receptor binding	Effect in relation to compound concentration	Dose-response characteristics
$T_R \rightarrow 0$	$C_R \sim C$	$T_r \rightarrow 0$	$E \sim C_R$	$E \sim C$	Dose-dependent
		$T_r \rightarrow \infty$	$E \sim \int C_R\, dt$	$E \sim \int C\, dt$	Ct=constant*
$T_R \rightarrow \infty$	$C_R \sim \int C dt$	$T_r \rightarrow 0$	$E \sim C_R$	$E \sim \int C\, dt$	Ct=constant
		$T_r \rightarrow \infty$	$E \sim \int C_R\, dt$	$E \sim \int \int C\, dt$	Reinforced by time

T_R is the time constant for the reversibility of receptor binding; T_r is the time constant for the reversibility of the effect; C_R is the concentration of bound receptors; C is the concentration of the poison at the site of interaction. E=Effect; *known as Haber's Rule (the product of concentration and time produces a constant effect)
Table 1: Dose-response characteristics according to Druckrey and Küpfmüller [26].

may not be associated with a threshold either [45-47]. The NRC panel concluded that linear models are most appropriate for dose–response modeling of mercury's neurodevelopmental effects in the absence of persuasive evidence supporting an alternative functional form [48].

Conclusion

Contrary to widely held belief, a threshold does not follow automatically from absence of genotoxic potential. The genotoxicity of an alkylating nitrosamine can be viewed as an example of irreversible receptor binding (covalent binding to DNA) associated with irreversible effects (gene mutations). Similar receptor-mediated mechanisms of toxic action are perfectly conceivable. Risk assessments should not assume thresholds for noncarcinogens as a matter of principle when there is mechanistic evidence of receptor-mediated toxicity [49]. *If the shape of the dose-response curve conveys a linear relationship between receptor occupancy and biological response at lower concentrations, a threshold may not exist.* This thesis constitutes a paradigm shift in a core area of toxicological sciences, but a linear dose-response relationship cannot and must not be ignored and should be point of departure for effective risk management. For such chemicals, risk management should be based on the ALARA principle ("as low as reasonably achievable") unless benefits clearly outweigh risks, for example with pharmaceuticals for treatment of cancer or other life-threatening diseases.

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