Development of a Dose-Response Model For Risk Assessment of Receptor-Mediated Effects

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1. Abstract

Two dose response models have traditionally been used in risk assessment. 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. Dose – response relationships of several neurotoxic non-carcinogens were recently shown to be identical to that of an alkylating carcinogen, and were theoretically explained by irreversible receptor binding with an associated irreversible effect. 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 as a matter of principle when there is mechanistic evidence of receptor-mediated toxicity. A dose response model for receptor-mediated toxicity needs to be developed, and 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. For chemicals with a linear dose-response relationship in low dose regions, risk management should be based on the ALARA principle ("as low as reasonably achievable").

2. 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 a threshold below which no adverse effects can be assumed to occur. The controversy is readily apparent in carcinogenic risk assessment. Analysis of chemical carcinogenicity and genotoxicity data generated by the U.S. National Toxicology Program (NTP) demonstrated that almost half of the chemicals that were positive in the bioassays for carcinogenicity were negative in genotoxicity tests (1). The dose-response model for carcinogenic risk assessment depends on absence or presence of genotoxic potential. 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.

3. The Controversial Linear Non-Threshold (LNT) Dose-Resp-onse Model For Carcinogenic Risk Assessment

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. In his Nobel Prize Lecture of December 12, 1946, Hermann J. Muller argued that the dose–response for radiation-induced germ cell mutations was linear and that there was *''no escape from the conclusion that there is no threshold''*(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. However, the LNT dose-response model has remained controversial and is not automatically employed for all genotoxic

substances on the other side of the Atlantic. 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-amino-biphenyl, diethylnitrosamine, acetamino-fluorene, aflatoxin B_1) or ionizing radiation (8). SCOEL does, however, also recognize genotoxic carcinogens for which the existence of a threshold cannot be sufficiently supported at present (acrylonitrile, acrylamide, arsenic) and genotoxic carcinogens with a practical threshold (formaldehyde, vinyl acetate). For the latter category health-based exposure limits may be based on an established NOAEL, whereas for the former category the LNT model is used as a default assumption, based on the precautionary principle (8). The LNT dose-response model was also 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. This prompted Calabrese to accuse the U.S. NAS of misleading the world community on cancer risk assessment (10-12) and to hypothesize that the most fundamental shape of the dose response may neither be threshold nor linear, but U-shaped (hormetic), and that hence both threshold and linearity models provide less reliable estimates of low-dose risk (13). 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 sub-stances, such as aflatoxin-like compounds, N-nitroso-compounds, and azoxy-comp-ounds, 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 ingre-dients, pharmaceutical manufacturing

operations (17, 18) and even for deriving target values for drinking water contaminants (19).

4. The Paradox: Non-Genotoxic Compounds May Behave Like Alkylating Carcinogens

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 essence of the common dose-response is that the total dose required to produce a defined effect decreases with time suggesting that effects are reinforced by time. From a mechanistic point of view, 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).

Table 1: Dose-response characteristics according to Druckrey and Küpfmüller (26)

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)

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

$$d\,t^{n} = \text{constant} \qquad (1)$$

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

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 insect-cides block nicotinic acetylcholine (nACh) receptors in the central nervous system of insects (30) which leads to irreversible neuronal damage (31), although Bayer CropScience scientists retracted this concept after Tennekes had pointed out the similarities with the mechanism of action of diethylnitrosamine (32). It is impossible to deny, however, that the affinity of imidacloprid for the nACh receptor in insects is very high, and that, unlike the normal neurotransmitter acetylcholine, acetylcholinesterase can not remove imidacloprid from the nACh receptor. 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). 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 an autocatalytic process initiated by binding of mercuric ion to sulfhydryl groups of organic macromolecules (23).

5. Current Risk Assessment Of Receptor-Mediated Effects is Flawed

As inferred earlier, two dose response models have traditionally been used in risk assessment. Most regulatory agencies assume that there is no safe level of exposure to carcinogens and use linear dose-response models to estimate human health risks at low exposure levels. In contrast, regulators usually assume that a

threshold, or "safe," exposure level exists for non-carcinogens. But why do we assume a threshold for non-carcinogens such as neonicotinoids and organic mercury and no threshold for the alkylating carcinogen diethylnitrosamine when their dose – response relationships are identical and their mechanisms of action show similarities and relate to irreversible receptor binding? At least one of the two dose response models must be flawed. There is now increasing evidence to suggest that the threshold model for non-carcinogens with time-cumulative toxicity may seriously underestimate actual risks.

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 (34, 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 is imperative.

6. A Dose-Response Model For Receptor-Mediated Effects

For identification of chemicals with time-cumulative toxicity, it is vital to perform toxicity testing geared to investigate the dose response of defined time-dependent effects. The Druckrey-Küpfmüller equation (1) with $n \geq 1$ can serve as a dose-response model for risk assessment of genotoxic and non-genotoxic compounds with time-cumulative toxicity. Carlborg (1981) pointed out that this equation is implied by a Weibull model for dose-response functions in carcinogenesis (39). Lucier et al (40), evaluating the effects of dioxins and pointing out that most, if not all, of dioxin's effects require interaction with a cellular protein, the Ah receptor, suggested that risk assessment should focus on the insights regarding dose-response relationships for receptor-mediated events and the application of this information to developing novel mathematical models that provide the foundation to use receptor mechanisms in risk assessment. The model needs to recognize the diversity of biological responses that are initiated by a single receptor interacting with a single ligand. In modeling biological phenomena, the data can be divided after Lucier et al. (40) into

four broad categories, as shown in Table 2 for effects observed with dioxins.

Table 2. Examples of levels of information available for estimating parameters in dose-response modeling, after Lucier et al (40).

Level	Parameter
Organism	Morbidity
	Mortality
	Fertility
	Improper development function
Tissue	Hyperplasia
	Hypertrophy
	Tumorigenesis
	Chemical distribution disposition
Cell	Mitosis
	Cell death
	Cytoarchitectural pathology
Biochemical	Gene expression
	Protein levels
	Receptor binding
	Adduct formation

The data range from very general (often no more detail than mortality data) to highly specific mechanistic data dealing with the interactions between molecules. At the top are effects on the whole animal. The levels of data then decrease and become more specific, going from whole organism to tissue/organ system responses to cellular responses, and finally down to biochemical responses in the cell. Evaluation of dose-response relationships for receptor-mediated events ultimately requires information on the quantitative relationships among ligand concentration, receptor occupancy, and biological response. A threshold response is possible for receptor-mediated effects, but it is not obligatory. Every toxin thought to exert its effects by binding to a specific receptor should be studied individually to determine its low-dose response. Dioxin is a case in point. 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). Similarly, the dose-response for promotion of liver tumor induction in mice by the non-genotoxic insecticide dieldrin was shown to be linear and consistent with the absence of a threshold (42). 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). Likewise, subtle effects on children's health of sulfhydryl-reactive metals, which include mercury (Hg), cadmium (Cd), lead (Pb), and arsenic (As), may not be associated with a threshold either (45,46,47). 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), 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.

7. References

1. Tennant RW, Margolin BR, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnik M, Stasiewicz S, Anderson B, Minor R. Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. Science 1987, 236: 933-941.
2. Bolt HM, Foth H, Hengstler JG, Degen GH. Carcinogenicity categorization of chemicals—new aspects to be considered in a European perspective. Toxicology Letters 2004, 151: 29–41
3. Anonymous. Genetic effects of atomic radiation. Summary Report of the Committee on Biological Effects of Atomic Radiation by the National Academy of Sciences, BEAR I Genetics Panel (W. Weaver, Chair). Science 1956, 124:170
4. Albert, RE. Carcinogen risk assessment. Environ Health Perspect 1989, 81: 103-105
5. Calabrese EJ. Muller's Nobel lecture on dose–response for ionizing radiation: ideology or science? Arch Toxicol 2011, 85: 1495–1498
6. Environmental Protection Agency (EPA). Health risk and economic impact assessments of suspected carcinogens. Fed Regist 1976, 41: 21402–21405
7. Environmental Protection Agency (EPA). Guidelines for Carcinogen Risk Assessment, 2005, EPA/630/P-03/001F, pp. 1–166
8. Bolt, HM, Huici-Montagud A. Strategy of the scientific committee on occupational exposure limits (SCOEL) in the derivation of occupational exposure limits for carcinogens and mutagens. Arch Toxicol 2008, 82:61–64
9. Thomas AD, Fahrer J, Johnson GE, Kaina B. Theoretical considerations for thresholds in chemical carcinogenesis, Mutat. Res.: Rev. Mutat. Res. 2015, http://dx.doi.org/10.1016/j.mrrev.2015.05.001
10. Calabrese, EJ. How the U.S. National Academy of Sciences mislead the world community on cancer risk assessment: New findings challenge historical foundations of the linear dose response. Arch Toxicol 2013 DOI:10.1007/s00204-013-1105-6
11. Calabrese EJ. An abuse of risk assessment: how regulatory agencies improperly adopted LNT for cancer risk assessment. Arch

Toxicol 2015 DOI 10.1007/s00204-015-1454-4

12. Calabrese EJ. The road to linearity: why linearity at low doses became the basis for carcinogen risk assessment. Arch Toxicol 2009, 83: 203–225
13. Calabrese EJ, Baldwin LA. Toxicology rethinks its central belief. Hormesis demands a reappraisal of the way risks are assessed. Nature 2003, 421: 691-692
14. Kroes R, Galli C, Munro I, Schilter B, Tran L.-A., Walker R,. Würtzen G. Threshold of toxicological concern for chemical substances present in the diet: A practical tool for assessing the need for toxicity testing. Food and Chemical Toxicology 2000, 38: 255–312
15. Kroes R, Kleiner J, Renwick A. The Threshold of Toxicological Concern Concept in Risk Assessment. Toxicol. Sci. 2005, 86 (2): 226-230.
16. van Ravenzwaay B, Dammann M, Buesen R, Schneider S. The threshold of toxicological concern for prenatal developmental toxicity. Regulatory Toxicology and Pharmacology 2011, 59: 81–90
17. Kroes R, Renwick AG, Feron, C, Galli, CL, Gibney M, Greim H, Guy RH, Lhuguenot JC, van de Sandt JJM. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food and Chemical Toxicology 2007, 45: 2533–2562
18. Dolan DG, Naumann BD, Sargent EV, Maier A, Dourson M. Application of the threshold of toxicological concern concept to pharmaceutical manufacturing operations. Regulatory Toxicology and Pharmacology 2005, 43: 1–9
19. Mons MN, Heringa MB, van Genderen J, Puijker LM, Brand W, van Leeuwen CJ, Stoks P, van der Hoek JP, van der Kooij D. Use of the Threshold of Toxicological Concern (TTC) approach for deriving target values for drinking water contaminants. Water Research 2013, 47: 1666 -1678
20. Tennekes, HA. The significance of the Druckrey–Küpfmüller equation for risk assessment - the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time. Toxicology 2010, 276: 1–4.
21. Tennekes HA, Sánchez-Bayo F. Time-dependent toxicity of neonicotinoids and other toxicants: Implications for a new approach to

risk assessment. Journal of Environmental & Analytical Toxicology 2012, S4:S4-001.

22. Tennekes HA, Sánchez-Bayo F. The molecular basis of simple relationships between exposure concentration and toxic effects with time. Toxicology 2013, 309: 39–51.
23. Pletz J, Sánchez-Bayo F, Tennekes HA. Dose-response analysis indicating time-dependent neurotoxicity caused by organic and inorganic mercury—Implications for toxic effects in the developing brain. Toxicology 2016 doi:10.1016/j.tox.2016.02.006
24. Magee PN, Barnes JM. The production of malignant primary hepatic tumours in. the rat by feeding dimethylnitrosamine. Brit. J. Cancer 1956, 10:114-22
25. Magee PN, Farber E. Toxic liver injury and carcinogenesis. Methylation of rat-liver nucleic acids by dimethylnitrosamine in vivo. Biochem J 1962, 83: 114-24.
26. Druckrey H, Küpfmüller, K. Dosis und Wirkung. Beiträge zur theoretischen Pharmakologie. Editio Cantor GmbH, Freiburg im Breisgau, Germany, 1949.
27. Druckrey H, Schildbach A, Schmaehl D, Preussmann R, Ivankovic S. Quantitative analysis of the carcinogenic effect of diethylnitrosamine. Arzneimittelforschung 1963, 13: 841–851
28. Druckrey H, Preussmann R, Ivankovic S, Schmahl D. Organotropic carcinogenic effects of 65 different N-nitroso-compounds in BD-rats. Z. Krebsforsch. 1967, 69:103-201
29. Sánchez-Bayo F. From simple toxicological models to prediction of toxic effects in time. Ecotoxicology 2009, 18: 343–354.
30. Abbink J, The Biochemistry of Imidacloprid. Pflanzenschutz-Nachrichten Bayer, Germany, F.R. 1991,Serial ID – ISSN: 0340-1723.
31. Mehlhorn H, Mencke, N, Hansen, O. Effects of imidacloprid on adult and larval stages of the flea *Cfenoaephalides felis* after in vivo and in vitro application: a light- and electron-microscopy study. Parasitol Res 1999, 85: 625-637
32. Maus C, Nauen R. Response to the publication: Tennekes, H.A. (2010): "The significance of the Druckrey-Küpfmüller equation for risk assessment: the toxicity of neonicotinoid insecticides to arthropods is reinforced by

exposure time". Toxicology 2011, 280: 176–177.

33. Tennekes HA. The significance of the Druckrey–Küpfmüller equation for risk assessment—the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time: responding to a Letter to the Editor by Drs. C. Maus and R. Nauen of Bayer CropScience AG. Toxicology2011, 280: 173–175.
34. Tennekes HA. The Systemic Insecticides: A Disaster in the Making. Zutphen, The Netherlands: ETS Nederland BV; 2010. Available from: http://www.disasterinthemaking.com/.
35. Morrissey CA, Mineau P, Devries JH, Sanchez-Bayo F, Liess M, Cavallaro MC, Liber K. Neonicotinoid contamination of global surfacewaters and associated risk to aquatic invertebrates: A review. Environment International 2015, 74: 291–303
36. Van Dijk TC, Van Staalduinen MA, Van der Sluijs JP Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid. PLoS ONE 2013, 8(5): e62374. doi:10.1371/journal.pone.0062374
37. Hallmann CA, Foppen RPB, van Turnhout CAM, de Kroon H, Jongejans, E, 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature 2014, 511: 341–343
38. Sorg M, Schwan H, Stenmans W, Müller A. Ermittlung der Biomassen flugaktiver Insekten im Naturschutzgebiet Orbroicher Bruch mit Malaise Fallen in den Jahren 1989 und 2013. Mitteilungen aus dem Entomologischen Verein Krefeld 2013, Vol. 1, pp. 1-5.
39. Carlborg FW. Dose-response functions in carcinogenesis and the Weibull model. Food Cosmet Toxicol 1981, 19: 255-63
40. Lucier GW, Portier CJ, Gall MA. Mechanisms and Dose-Response Models for the Effects of Dioxins. Environ Health Perspect 1993, 101: 36-44
41. Melnick RL, Kohn MC, Portier CJ. Implications for Risk Assessment of Suggested Nongenotoxic Mechanisms of Chemical Carcinogenesis. Environ Health Perspect 1996, 104 (Suppl 1):123-134
42. Tennekes H, van Ravenzwaay B, Kunz HW. Quantitative aspects of

enhanced liver tumour formation in CF-1 mice by dieldrin. Carcinogenesis 1985, 6: 1457-1462

43. Sheehan DM, Willingham E, Gaylor D, Bergeron JM, Crews D. No Threshold Dose for Estradiol-induced Sex Reversal of Turtle Embryos: How Little Is Too Much? Environ Health Perspect 1999, 107: 155-159
44. Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS. Large Effects from Small Exposures. I. Mechanisms for Endocrine-Disrupting Chemicals with Estrogenic Activity. Environ Health Perspect 2003, 111:994–1006
45. de Burbure C; Buchet J-P; Leroyer A; Nisse, C; Haguenoer, J-M ; et. al. Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. In: Environ Health Perspect 2006, 114(4): 584-90
46. Bernard A, Thielemans N, Roels H, Lauwerys R. Association between NAG-B and cadmium in urine with no evidence of a threshold. Occup Environ Med 1995, 52: 177-180
47. Quig D. Cysteine Metabolism and Metal Toxicity. Altern Med Rev 1998; 3(4): 262-270.
48. NRC (National Research Council). Toxicological Effects of Methylmercury. Washington, DC: National Academy Press, 2000.
49. Wigle DT, Lanphear BP Human Health Risks from Low-Level Environmental Exposures: No Apparent Safety Thresholds. PLoS Med 2005, 2(12): e350. doi:10.1371/journal.pmed.0020350