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Review

Threshold of Toxicological Concern for Chemical Substances Present in the Diet: A Practical Tool for Assessing the Need for Toxicity Testing

R. KROES[1], C. GALLI[2], I. MUNRO[3], B. SCHILTER[4], L.-A. TRAN[5]*, R. WALKER[6] and G. WÜRTZEN[7]

[1]RITOX–Utrecht University, Faculty of Veterinary Medicine, PO Box 80176, 3508 TD Utrecht, The Netherlands, [2]University of Milan, Institute of Pharmacological Sciences, Via Balzaretti, 9, 20133 Milan, Italy, [3]CanTox Inc., 2233 Argentia Road, Suite 308, L5N 2X7 Mississauga, Ontario, Canada, [4]Nestec Ltd, Nestlé Research Centre, Case Postale 44, Vers-Chez-Les-Blanc 1000, Lausanne 26, Switzerland, [5]ILSI Europe, Avenue E. Mounier 83, Box 6, 1200 Brussels, Belgium, [6]University of Surrey, School of Biological Sciences, Guildford, Surrey GU2 5XH, UK and [7]Coca-Cola Nordic and Northern Eurasia Division, Hovedvejen 91, 2, sal, 2600 Glostrup, Denmark

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Introductory Note—The present paper was extensively discussed during a Workshop on "Threshold of Toxicological Concern for Chemical Substances Present in the Diet", held in Paris on 5–6 October 1999. A report of this meeting will be published in the very near future. For more detailed information, please contact the corresponding author.

Executive Summary—The *de minimis* concept acknowledges a human exposure threshold value for chemicals below which there is no significant risk to human health. It is the underlying principle for the US Food and Drug Administration (FDA) regulation on substances used in food-contact articles. Further to this, the principle of Threshold of Toxicological Concern (TTC) has been developed and is now used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in their evaluations. Establishing an accepted TTC would benefit consumers, industry and regulators, since it would preclude extensive toxicity evaluations when human intakes are below such threshold, and direct considerable time and cost resources towards testing substances with the highest potential risk to human health. It was questioned, however, whether specific endpoints that may potentially give rise to low-dose effects would be covered by such threshold.

In this review, the possibility of defining a TTC for chemical substances present in the diet was examined for general toxicity endpoints (including carcinogenicity), as well as for specific endpoints, namely neurotoxicity and developmental neurotoxicity, immunotoxicity and developmental toxicity. For each of these endpoints, a database of specific no-observed-effect levels (NOELs) was compiled by screening oral toxicity studies. The substances recorded in each specific database were selected on the basis of their demonstrated adverse effects. For the neurotoxicity and developmental neurotoxicity databases, it was intended to cover all classes of compounds reported to have either a demonstrated neurotoxic or developmentally neurotoxic effect, or at least, on a biochemical or pharmacological basis were considered to have a potential for displaying such effects. For the immunotoxicity endpoint, it was ensured that only immunotoxicants were included in the database by selecting most of the substances from the Luster *et al.* database, provided that they satisfied the criteria for immunotoxicity defined by Luster. For the developmental toxicity database, substances were selected from the Munro *et al.* database that contained the lowest NOELs retrieved from the literature for more than 600 compounds. After screening these, substances showing any effect which could point to developmental toxicity as broadly defined by the US EPA (1986) were recorded in the database.

Additionally, endocrine toxicity and allergenicity were addressed as two separate cases, using different approaches and methodology.

The distributions of NOELs for the neurotoxicity, developmental neurotoxicity and developmental toxicity endpoints were compared with the distribution of NOELs for non-specific carcinogenic endpoints.

*Corresponding author. Tel: 32-2-771 00 14; Fax: 32-2-762 00 44.

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As the immunotoxicity database was too limited to draw such a distribution of immune NOELs, the immunotoxicity endpoint was evaluated by comparing immune NOELs (or LOELs—lowest-observed-effect levels—when NOELs were not available) with non-immune NOELs (or LOELs), in order to compare the sensitivity of this endpoint with non-specific endpoints.

A different methodology was adopted for the evaluation of the endocrine toxicity endpoint since data currently available do not permit the establishment of a clear causal link between endocrine active chemicals and adverse effects in humans. Therefore, this endpoint was analysed by estimating the human exposure to oestrogenic environmental chemicals and evaluating their potential impact on human health, based on their contribution to the overall exposure, and their estrogenic potency relative to endogenous hormones.

The allergenicity endpoint was not analysed as such. It was addressed in a separate section because this issue is not relevant to the overall population but rather to subsets of susceptible individuals, and allergic risks are usually controlled by other means (i.e. labelling) than the Threshold of Toxicological Concern approach. However, as several researchers are currently examining the existence of a threshold in allergy, the possibility of determining threshold doses for food allergens was put into perspective, and the likelihood for chemical substances to induce allergy at dietary relevant doses was discussed.

The analysis indicated that, within the limitation of the databases, developmental neurotoxicity and developmental toxicity were not more sensitive than other non-specific endpoints.

Although the cumulative distribution of NOELs for neurotoxic compounds was significantly lower than those for other non-cancer endpoints, these substances were accommodated within the TTC of 1.5 μg/person/day. Furthermore, the analysis demonstrated that none of the specific non-cancer endpoints evaluated in the present study was more sensitive than cancer and, that a TTC of 1.5 μg/person/day based on cancer endpoints provides an adequate margin of safety.

Analysis of the immunotoxicity database showed that for the group of immunotoxicants examined here, the specific immunotoxic endpoint was not more sensitive than other endpoints. In other words, the distribution of immunotoxic NOELs for these compounds did not appear to differ from the distribution of non-specific endpoints NOELs for the same compounds.

The dietary intakes of environmental oestrogenic chemicals were estimated and their oestrogenic potencies were compared with that of endogenous hormones, in order to assess their impact on human health. The results are in line with scientific data obtained so far, suggesting that estrogenic compounds of anthropogenic origin, in comparison with endogenous hormones, possess only little hormonal activity like phytoestrogens. Results of animal studies do not suggest that hormonal effects are to be expected from the rather low concentrations found in foods.

More data are necessary to determine threshold doses for food allergens. However, provided that numerous criteria need to be satisfied before sensitization occurs, it is unlikely that small molecules used in little amounts in foods would induce such reactions.

On the basis of the present analysis, which was conducted using conservative assumptions at each step of the procedure (i.e. in data compilation and data analysis), and continually adopting a "worst case" perspective, it can be concluded that a Threshold of Toxicological Concern of 1.5 μg/person/day provides adequate safety assurance. Chemical substances present in the diet that are consumed at levels below this threshold pose no appreciable risk.

Moreover, for compounds which do not possess structural alerts for genotoxicity and carcinogenicity, further analysis may indicate that a higher Threshold of Toxicological Concern may be appropriate. © ***2000 Elsevier Science Ltd. All rights reserved***

Keywords: threshold; toxicological endpoints; structural alerts; NOEL.

Abbreviations: ADI = acceptable daily intake; CPD = carcinogenic potency database; CTL = cytotoxic T-lymphocyte; DBPCFC = double-blind, placebo-controlled food challenge; EE = Estrogen Equivalents; FDA = Food and Drug Administration; LOEL = lowest-observed-effect level; MTD = maximum tolerated dose; NK = natural killer; NOAEL = no-observed-adverse-effect level; NOEL = no-observed-effect level; PFCs = plaque-forming cells; RA = risk assessment; RPE = relative proliferative effect; TDI = tolerable daily intake; TTC = Threshold of Toxicological Concern.

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Introduction

Humans are exposed to thousands of chemicals, either naturally occurring or man-made. This review has been prepared with the aim of assessing whether a generic threshold value or range of values can be established, which may preclude the need for extensive and normally expensive toxicity studies and safety evaluations when human intakes are below such established thresholds. Some general remarks are made concerning the world of chemicals, toxicity testing and safety evaluation, the principle of a threshold and differing risk assessment philosophies in different countries or regions. Special consideration is given to the possibility of defining a threshold of toxicological concern for general toxicity endpoints (including carcinogenicity) as well as for the specific endpoints neurotoxicity and developmental neurotoxicity, immunotoxicity and developmental toxicity. Additionally, attention is given to endocrine toxicity and allergenicity, which are also matters of concern when evaluating possible adverse reactions of substances.

The world of chemicals

It has been estimated that there are over five million man-made chemicals known, of which only a limited number (approximately 70,000) are in commercial use today (Beck *et al.*, 1989). Furthermore, it has been reported that there are more than 100,000 naturally occurring substances of known structure, but it can be assumed that many more exist, the structures of which are not yet elucidated.

Humans are exposed to thousands of chemicals every day by different routes, each having their own barrier system. Effects through exposure by inhalation are determined by the properties of a chemical, for example its molecular weight, relative volatility and/or particle size. The chemicals that reach the alveolar space still have to pass the alveolar lining before entering the circulatory system of the body. Similarly, effects through dermal exposure to substances may—apart from chemicals reacting directly with the dermal epithelium—depend on the properties of a chemical to penetrate the skin. Finally, the entry of chemicals into the human body by oral exposure is dependent on the ability of the chemical or its breakdown products to pass the gastrointestinal lining and to enter the body via the lymph or portal venous system.

When considering substances migrating into food from packaging materials or other dietary sources, apart from the normal food constituents and the naturally occurring substances in food, particularly vegetables and fruits (NRC, 1996), a large number of food additives or many other substances are in use. Materials coming into contact with food may add another 3000 substances (Hayes and Campbell, 1986). A similar number of substances are used in flavourings. Evidently not all substances are present at the same time and, similarly, the levels of use and subsequently the levels of exposure for humans is extremely low for many substances. For example, the vast majority of food additives are present in small amounts, especially in regard to so-called ''indirect food additives''. One does not have to argue that it is impossible to subject all the chemical substances to which humans are exposed to extensive toxicological testing. Furthermore, if sufficient facilities to perform such testing within a reasonable time were available, it still can be questioned whether testing of all these substances would be a rational and practical approach. Therefore, the establishment of a scientifically based generic threshold will be a useful tool to discern which substances of concern should be subjected to elaborate testing, when human intake is higher than the generic threshold.

Toxicity testing and safety evaluations

In the past four decades toxicity testing has grown to maturity and today a systematic, usually tiered approach is used to establish whether adverse effects occur and if so, to investigate at what levels of exposure such adverse effects remain absent, and whether a dose–response relationship can be established. On the basis of these findings, a safety evaluation may be performed to assess at what levels of exposure humans may not experience any risk. The basis of such an evaluation is usually the established no-observed-effect level (NOEL) in animal testing (WHO, 1987) also referred to as an experimental threshold.

Threshold

Although the word ''threshold'' in classical pharmacology is used to define a level above which a desired effect is seen, in toxicology a threshold is defined as a dose at, or below which, a response is not seen in an experimental setting. Establishing proof of absence of an effect at such a dose in absolute terms is scientifically and practically not feasible since only a limited number of experimental animals will be used for practical and economic reasons.

Additional means, which are used sometimes to establish toxicological thresholds, are mechanistic and biotransformation information. The threshold principle is based on the assumption that at or below that threshold, homeostasis is maintained. This is, in essence, true for almost all toxicological endpoints, with the exception of genotoxic carcinogens where, for regulatory purposes, it is often assumed that the threshold does not exist.

Approaches to safety evaluation and risk assessment

Although in many cases current risk assessment (RA) is based on relatively simple safety assessments, more advanced systems have been proposed and are also in use for specific endpoints. These new RA methodologies are directed to assess the

quantitative risks rather than a qualitative assessment of risk, the latter leading to the provision of absence of an appreciable health risk.

For example, when the Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimates ADIs (acceptable daily intakes) or TDIs (tolerable daily intakes), their evaluation is based on toxicological concern, which means considering the type of effects, the potency, and the relevance for humans. The ADI* is calculated from the NOEL† in animal studies, adjusted with a safety factor.

More than 30 years of practice with this qualitative approach has shown its usefulness for regulatory purposes. Safety evaluation according to the ADI concept can be applied for all kinds of toxicological effects for which a threshold is assumed. It does not provide a quantification of the risk. However, the period of time over which this method has been used without any manifestation of toxic effects arising from the use of compounds managed in accordance with the ADI concept suggests that the resulting actual risk is probably very low.

As stated earlier, quantitative RA has been developed in recent years and is used particularly in the RA of carcinogenic substances and methods have been recognized internationally. However, classification of carcinogens into two main categories—genotoxic and non-genotoxic substances—for the purpose of assessing risk is not harmonized internationally. In general, genotoxic carcinogens are regarded as non-threshold toxicants with risk at all dose levels, whereas non-genotoxic carcinogens are regarded as threshold toxicants with no risk at sufficiently low doses.

In quantitative RA of carcinogens which are genotoxic, several mathematical models have been proposed. Such models are usually based on the assumption that a linear relationship exists between the quantity of exposure and the response for the particular endpoint. The use of such quantitative cancer RA models has given cause for a number of generic and non-generic factors, which may increase the calculated cancer risk considerably due to using the worst case approach. For example, the policies concerned include among others factors weight *vs* surface area; maximum or average likelihood *vs* upper 95% confidence; malignant *vs* malignant plus benign tumours; average animal sensitivity *vs* most sensitive animal; pharmacodynamics *vs* effective dose. A combination of all the above default options might in the worst case increase the estimated cancer risk up to 10,000 times (Barnard, 1994). Thus, the estimated risk may be unrealistically higher by orders of magnitude and this may need further consideration.

Moolenaar (1994) has compared the methods and approaches used internationally in RA of carcinogens. He did not find any country in which estimated cancer risks systematically were placed in context with other risks. He concluded "The US EPA has established the estimation of an upper limit to carcinogen risk as a goal for risk assessment, while European counterparts have established the estimation of the likely incidence of cancer in the human population as the goal for risk assessment. The US approach depends heavily on conservative generic default assumptions to bridge areas of uncertainty and derive estimates of the upper limit to risk. The European approach favours case-by-case application of scientific judgements to resolve uncertainties and derive estimates of the expected incidence of cancer in the human population".

The concept of a threshold of toxicological concern

The concept that there is a level of exposure to a given substance below which no significant risk is expected to exist has been widely accepted, and as depicted earlier, the establishment of ADIs is based on that concept (SCF, 1996; WHO, 1987).

Frawley (1967) was the first to present an analysis to establish a generic threshold value (threshold of regulation) or range of values with the aim to reduce extensive toxicity studies and safety evaluations, and to address, within the available capacity, those substances for which the potential or actual intake is substantial. In 1986, Rulis conducted a similar analysis of the FDA's Priority-Based Assessment of Food Additives (PAFA) database containing 159 compounds with subchronic or chronic toxicity data, LD_{50} values from 18,000 oral rodent studies contained in the Registry of Toxic Effects of Chemical Substances (RTECS), and TD_{50} values for 130 compounds found in the carcinogen potency database of Gold *et al.* (1984). Both scientists concluded that an intake for humans between 1 and 10 μg/kg body weight/day of various chemical substances might not pose a risk to humans.

Munro *et al.* (1996) compiled a large database of reference substances from which a distribution of NOELs could be derived. This database includes structure and toxicity endpoints for a wide variety of organic chemicals, selected according to strict criteria. Three classes according to Cramer *et al.* (1978) were identified:

- Class I: Substances of simple chemical structure and efficient modes of metabolism which would suggest a low order of oral toxicity.
- Class II: "Intermediate" substances which possess structures that are less innocuous than class I substances but do not contain structural features

*ADI: An estimate by JECFA of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk (standard man = 60 kg) (WHO, 1987).

†NOEL: The greatest concentration or amount of an agent, found by study or observation, that causes no detectable, usually adverse, alteration of morphology, functional capacity, growth, development, or lifespan of the target (WHO, 1987).

suggestive of toxicity like those substances in class III.

- Class III: Substances of a chemical structure that permits no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.

The database includes the usual types of toxicity studies (subchronic, chronic, reproductive and teratology studies) used for evaluation. The database mainly consists of studies in rodents and rabbits. Dog and monkey studies were not included since many had too few animals per group to derive statistically valid NOELs, were too short in duration or were hampered by insufficiencies originating from palatability problems of the diet. For each substance with a defined NOEL, an LOEL was also included. When chronic NOEL data were not available, they were compiled from the NOEL data from subchronic studies. These were divided by a factor of three, based on a retrospective analysis performed by others (Beck *et al.*, 1993; Lewis *et al.*, 1990; Weil and McCollister, 1963). NOELs selected were the NOELs suggested by the authors, even though they were in a number of cases over-interpretations of their data. However, obvious misjudgements were corrected.

The database described above was used to calculate human exposure thresholds for the three structural classes identified, using the 5th centile of the distributions of NOELs divided by an uncertainty factor of 100. Human exposure levels were respectively 1800, 540 and 88 μg/person/day for classes I, II and III.

The procedure described above was also the basis for a procedure for safety evaluation of flavouring substances described by Munro and Kroes (1998) and Munro *et al.* (1999). In this procedure, in addition to the derivation of human exposure limits by class, other endpoints including cancer were evaluated. For the cancer endpoint, the carcinogenic potency database (CPD) as originally compiled by Gold *et al.* (1984) and further updated (Gold *et al.*, 1989) was used. In a workshop organized by Munro (1990), factors that influence the selection of an appropriate threshold value for carcinogens were evaluated. It was concluded that when using a threshold of 1.5 μg/person/day, the probability of exceeding a risk of 10^{-6} for new chemicals entering the database was low, especially when it was assumed that less than 50% of such new chemicals would be potential carcinogens. Eliminating substances with structural alerts for carcinogenicity would reduce the probability considerably (Ashby and Tennant, 1988, Tennant and Ashby, 1991).

In the papers of Munro and Kroes (1998) and Munro *et al.* (1999), other selected endpoints such as reproductive effects and neurotoxicity were assessed as well in the Munro database, and immunotoxicity was assessed by evaluating a limited number of immunotoxic chemicals from the Luster database (Luster *et al.*, 1992, 1993). For developmental abnormalities and neurotoxic compounds, human exposure threshold values were again calculated using the 5th centile NOEL divided by an uncertainty factor of 100, and assuming an average individual weight of 60 kg per person. The various human exposure threshold values are given below:

Various human exposure threshold values (Munro and Kroes, 1998)

	5th Centile NOEL (mg/kg body weight/day)	Human exposure threshold (μg/person /day)
Structural class I	3	1800
Structural class II	0.91	540
Structural class III	0.15	88
Developmental abnormalities	3.46	2076
Neurotoxicity	0.03	18

For immunotoxicity, an insufficient number of substances was available to calculate a reliable 5th centile NOEL. Therefore, a comparison was made of non-immunotoxic NOEL *vs* immunotoxic NOEL, and similarly for the LOEL. It was noticed that usually the non-immunotoxic NOEL and LOEL were lower but that in the cases when immunotoxic NOELs (and LOELs) were lower, this was less than 10-fold lower than the non-immunotoxic counterpart.

The concern was raised by others that a derivation of the 5th centile NOEL based on the currently used databases drawn from acute, subchronic and chronic studies in rodents may not adequately cover endpoints which might give rise to important low dose effects such as neurotoxic, immunotoxic, endocrinologic and developmentally toxic events (SCF, 1996). Therefore, it was decided to undertake the present study to further assess human exposure threshold values for the specific endpoints neurotoxicity and developmental neurotoxicity, immunotoxicity and developmental toxicity, and to examine these endpoints in order to determine whether changes in the different parameters of these specific systems would occur at particularly low levels of exposure, and what these levels would be. The approach involved the collection of data pertaining to NOELs for these various endpoints, and the assessment as to whether the distributions for the various NOELs differed significantly from the distributions for general toxicity as described by Munro *et al.* (1996, 1999).

In addition, special attention has been given to endocrine effects and allergenicity which are presently major subjects of public concern. Both issues are matters to be considered when evaluating possible adverse reactions to chemical substances, but it was decided to address them as separate cases.

Usually, traditional reproductive studies cover endocrine substances, but since endocrine toxicity has become a public concern and scientific claims have been raised about the potential oestrogenic activity of certain environmental chemicals, a specific evaluation of this endpoint was undertaken. A different methodology was adopted for evaluating the endocrine toxicity endpoint since currently available data do not permit the establishment of a clear causal link between endocrine disrupting chemicals and adverse effects in humans. No specific database on endocrine toxicity was built up because it was not possible to compile substances on the basis of their demonstrated specific adverse effect, as was done for the other endpoints. Therefore, the approach adopted in this case was to estimate the possible human exposure to environmental oestrogenic chemicals, to relate their potencies to that of endogenous hormones and assess the potential impact of oestrogenic environmental chemicals on human health.

Allergenicity was confined in a separate section of the paper because this issue is not relevant to the overall population but rather to subsets of susceptible individuals within the population. As a consequence, allergic risks are usually controlled by other means (i.e. labelling) than the Threshold of Toxicological Concern approach. However, as several researchers are currently examining the existence of a threshold in allergy, it was felt important to put this case into the context of our evaluation.

Materials and Methods

Criteria for selecting specific endpoints and parameters

Although there exist testing guidelines designed for assessing specific toxicity as part of the general toxicological profile (e.g. assessment of toxic effects of direct food and colour additives on the nervous system) (FDA, 1982), the information derived from screening studies conducted according to these guidelines is usually limited to the detection of evident effects in adults (e.g. gross neuropathology). It was felt necessary, therefore, to carefully select the parameters to be recorded for each endpoint, in order to ensure the most sensitive and specific examination of the neurotoxicity, immunotoxicity and developmental toxicity endpoints.

Neurotoxicity endpoint. Neurotoxicity refers to any adverse effect of exposure to chemicals on the structure or functional integrity of developing or adult nervous systems. In the process of evaluating the safety of food components, the traditional approach to neurotoxicity is based on major neurological dysfunction supported by gross morphological changes and pathological lesions. Concerns have recently been raised about very subtle but important types of neurotoxic effects, including behavioural dysfunction. Furthermore, unlike most organ systems, the nervous system develops throughout gestation, exhibiting spurts of morphological or biochemical development which can be of

Table 1. (from Luster *et al*., 1992) Panel for detecting immune alterations following chemical or drug exposure in rodents[1]

Procedures	Reference for original method	Reference for NTP modification[2]
Tier I		
Haematology (e.g. leucocyte counts)		
Weights—body, spleen, thymus, kidney, liver		
Cellularity—spleen, bone marrow		
Histology of lymphoid organ		
IgM antibody plaque-forming cells (PFCs)	Cunningham *et al*., 1965	Dean *et al*., 1989
Lymphocyte blastogenesis	Anderson *et al*., 1972	Dean *et al*., 1989
T cell mitogens (PHA, Con A)	Bach and Voynow, 1966	
T cell (mixed leucocyte response MLR)		
B cell (lipopolysaccharide, LPS)		
Natural killer cell activity	Brunner *et al*., 1976	
Tier II		
Quantitation of splenic B and T lymphocytes (surface markers)		
Enumeration of IgG antibody PFC response[3]		
Cytotoxic T lymphocyte (CTL) cytolysis or delayed hypersensitivity response (DHR)	Lefford, 1974	Hosalppie *et al*., 1984
Host resistance[4]		
Syngeneic tumour cells		Murray *et al*., 1985
PYB6 sarcoma (tumour incidence)		
B16F10 melanoma (lung burden)		
Bacterial models		Bradley *et al*., 1985
Listeria monocytogenes (morbidity)		
Streptococcus species (morbidity)		
Viral models		
Influenza (morbidity)		
Parasite models		
Plasmodium yoelii (parasitemia)		

[1]The testing panel was developed using B6C3F1 mice. [2]Given if modifed from original reference. [3]Only a limited number of tests were conducted for this response and are not included in the present analysis. [4]For any particular chemical tested only one or two host resistance models were selected for examination.

particular vulnerability to various toxins. These make the examination of non-conventional parameters including behavioural endpoints, neurochemical changes or developmental neurotoxic effects becoming a requirement in neurotoxicity evaluation. For example, OECD Guidelines for 13-week oral studies adopted in 1998 contain extensive neurotoxicity testing requirements and methods. The US FDA has also proposed to focus its revised toxicity testing guidelines on behavioural and developmental neurotoxic effects, where the results of the first traditional tests indicated a potential for neurotoxicity.

The parameters recorded for the following evaluation of the neurotoxicity endpoint were therefore selected because of their possible higher sensitivity, although not necessarily leading to a functional deficit (acetylcholinesterase inhibition, neurochemical fluctuations) or their ability to reflect a global functional effect (behavioural alterations).

Immunotoxicity endpoint. Although there is presently no established definition of an immunotoxic substance, most agree that immunotoxicity can be defined as the adverse effects of foreign substances on the immune system that may result either in immuno-suppression or in immuno-potentiation (Luster *et al*., 1992). Currently-used methods for testing effects on the immune system measure conventional parameters such as alterations in lymphoid organ weight or histology, quantitative changes in peripheral leucocyte counts and differentials, depressed cellularity of lymphoid tissues (Dean and Murray, 1990; Trizio *et al*., 1988). Many agree that any change in conventional parameters which points to immunotoxicity should be investigated in detail by performing immune function tests including parameters such as: IgM antibody plaque-forming cells (PFCs), lymphocyte blastogenesis (T and B cells), natural killer (NK) cell activity, quantitation of splenic B and T lymphocytes (surface markers), enumeration of IgG antibody PFC response, cytotoxic T lymphocyte (CTL) cytolysis, delayed hypersensitivity response (DHR), host resistance to tumour cells, bacteria, virus or parasite (Luster *et al*., 1992, 1993; Trizio *et al*., 1988).

Therefore, the parameters recorded and the criteria of immunotoxicity used in the following evaluation of the immunotoxicity endpoint are those described by Luster *et al*. (1992, 1993). This was the only systematic approach available and additional compounds were evaluated in a comparable manner. According to these criteria, a substance is classified as immunotoxic when the test produces a significant dose–response effect ($P < 0.05$) or significantly ($P < 0.05$) altered two or more test parameters (Table 1) at the highest dose of chemical tested.

Developmental toxicity endpoint. Developmental toxicity is broadly defined in the Guidelines for the Health Assessment of Suspect Developmental Toxicants (EPA, 1986). Moreover, evidence is accumulating which indicates the need for postnatal evaluation of systems other than just the reproductive system (Roberts and Chapman, 1981). Consequently, the parameters examined in the following evaluation of the developmental toxicity endpoint were not restricted to birth defects or teratological manifestations, but also included various effects on the developing organism such as resorptions, intrauterine and perinatal deaths, structural abnormalities, altered growth, and functional deficits. Special attention was also given to parameters such as postnatal growth, neonatal survival, and viability of prenatally exposed offspring which are used as a measure of developmental toxicity (Chernoff and Kavlock, 1982), since experience has shown that terata occurs only rarely compared with other parameters of developmental toxicity and at higher doses (Ulbrich and Palmer, 1996; Vorhees, 1987). In addition, early lethality was recorded because lethality is considered to be related to developmental effects when occurring within the first month after birth. Obviously, gross morphological and structural changes were recorded. Conversely, food consumption data, and effects considered to be directly related to these parameters were not included in the database because they were not considered to be specifically indicative of developmental toxicity.

Criteria for selecting substances and compilation of the specific databases

For the analysis of specific endpoints, chemical substances evaluated in this report were selected on the basis of their demonstrated specific adverse effects. This selection procedure probably overestimates the proportion of chemicals displaying such adverse effects as a major determinant of risk. Toxicological data on these substances were drawn from peer-reviewed scientific literature or authoritative sources such as JECFA or the US EPA.

Neurotoxicity database. For the neurotoxicity endpoint evaluation, an attempt was made to cover all classes of substances reported to have either a demonstrated neurotoxic or developmentally neurotoxic effect or at least, on a biochemical or pharmacological basis, was considered to have a potential for displaying such effects.

Compilation of the neurotoxicity database started by a preliminary screening through general reference bibliography (Anthony and Graham, 1990) and several review articles (Desi, 1983; Goldey *et al*., 1995) that permitted the listing of most of the best characterized and documented classes of neurotoxicants and developmental neurotoxicants. In addition to these compounds, other substances with reported neurotoxic NOELs from the Munro *et al*. (1996) database were included. Whenever possible, additional substances were added in the neurotoxicity database. Original papers and additional

studies were retrieved through MEDLINE, the US EPA Integrated Risk Information System (IRIS), the toxicological monographs prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the toxicological monographs prepared by the Joint Meeting on Pesticides Residues (JMPR), and other chemical and toxicological databases on CD-ROM (*Sax's Dangerous Properties of Industrial Materials, Hawley's Condensed Chemical Dictionary*).

Considering that the developing nervous system may be more sensitive to neurotoxicants than that of adults, it is usually expected that neurotoxic effects elicited in the developing organisms occur at lower dose ranges than in adults (NTIS, 1991). Moreover, studies conducted to detect developmental neurotoxicity differ in many aspects from those aimed at examining neurotoxicity in adults (e.g. one of the major differences is that in neurodevelopmental studies, dams are exposed to treatment but effects are observed in the offspring. That is to say the offspring is exposed via transplacental route, and actual delivered treatment doses are unknown) (Ulbrich and Palmer, 1996). Consequently, two separate databases were developed. The Neurotoxicity Database (Annex 1) focuses on neurotoxicity in adults and contains 82 substances (37 acute + 45 chronic or subchronic studies). The Developmental Neurotoxicity Database (Annex 2) focuses on neurotoxicity in developing organisms and contains 52 substances (teratogenicity or reproduction studies). In this evaluation, studies and substances will be included in each database according to the following definitions:

- Neurotoxicity: the toxic effects affecting the nervous system in adult organisms.
- Developmental neurotoxicity: the toxic effects affecting the nervous system following exposure *in utero* or during the immediate post-natal period.

Immunotoxicity database. For the immunotoxicity endpoint evaluation, the majority of substances examined were drawn from the Luster *et al*. (1992, 1993), database among which 24 immunotoxins had been previously evaluated by Munro and Kroes (1998) and Munro *et al*. (1999). All the selected substances satisfied the criteria for immunotoxicity defined by Luster *et al*. (1992, 1993). It was therefore ensured that only immunotoxicants were included in the immunotoxicity database.

As the Luster database consisted of studies conducted from 1980 to 1992, the literature was screened in order to check whether more recent studies existed for the same substances. When a lower NOEL was retrieved, we kept the latest study and removed the Luster database NOEL (when a NOEL from Luster data was replaced, the new NOEL is indicated by "§"). As for the neurotoxicity database, additional substances retrieved from MEDLINE, IRIS, JECFA or JMPR reports were added in the immunotoxicity database, whenever possible. Overall, the evaluation of the immunotoxicity endpoint was carried out on a total of 37 substances positively classified as immunotoxins (Annex 3).

Developmental toxicity database. All the substances examined in the developmental toxicity endpoint evaluation were taken from the Munro *et al*. (1996) database which was chosen as a starting point because of its convenience (i.e. selection of the studies conducted in rodents, oral route of exposure, lowest NOELs from the literature). No attempt was made to restrictively select substances with reported or characterized teratological and developmental effects, but rather to screen for any effect which could point to developmental toxicity as broadly defined by the US EPA (1986). By keeping all the reproductive studies, as well as the studies where reported endpoints were teratological, and after going back to the original papers, it was expected to record reproductive and developmental effects occurring at the lowest levels identified in the literature for each substance. Globally, 81 NOELs for developmental toxicity were compiled (Annex 4).

Criteria for selecting toxicological studies and NOELs

Priority was placed on studies where the route of exposure was oral administration (gavage, feeding or drinking water), but in a few cases the treatment was given by non-oral administration (e.g. injection route in the case of pharmaceutical agents).

The majority of the databases consists of toxicity studies conducted in rodents but in a few cases, other species were included. For example, hens are usually tested in studies of delayed neurotoxicity (induced by certain organophosphate insecticides) since they are known to best reproduce the syndrome observed in humans.

When doses were not provided in mg/kg body weight/day, conversions were calculated by using standard values (available from the US EPA). In one study from the immunotoxicity database, the route of exposure to arsine was inhalation. Therefore, the exposure concentrations were converted from ppm to absorbed doses expressed in mg/kg body weight/day, according to indications from the US EPA IRIS database.

When more than one NOEL was identified for the selected parameters (e.g. neurobehavioural, neurochemical, neurodevelopmental, immune parameters or developmental endpoints), the lowest NOEL was retained. A real effort was made to select studies with a demonstrated LOEL as well as a NOEL to ensure that the study was rigorous enough to detect toxic effects. However, most of the studies were special neurotoxicity or immunotoxicity studies aimed at identifying effects and were not necessarily designed to determine a dose–response relationship. Also, because the administered substances were generally well-known toxicants, the

active doses of which are documented, the ranges of doses tested were sometimes quite narrow and did not provide definitive data on the LOEL and NOEL.

Setting the worst-case scenario

The selection of the parameters, substances and NOELs to be analysed in the present evaluation was carried out using a number of conservative assumptions, as explained earlier. Following such an approach in the evaluation of every specific endpoint assured that substances and studies were not randomly included in each database, but were compiled in order to set the worst case situation when assessing each specific endpoint.

Neurotoxicity endpoint. In the evaluation of the neurotoxicity endpoint, neurotoxic and developmentally neurotoxic substances were retrieved, as described above, by first consulting a reference bibliography and listing all classes of known toxicants of the nervous system. Among such toxicants, a large number of molecules are especially designed to be highly potent neurotoxins (65 pesticides out of 82 substances in the neurotoxicity database), or specifically active on the nervous system (14 neuroactive agents and drugs out of 52 substances in the developmental neurotoxicity database), so that the final database is heavily weighted to those substances with the highest neurotoxic potential, which hardly corresponds to real situation but rather to a worst case scenario. In addition, a careful selection of the neurotoxic parameters thought to be the most sensitive and subtle (i.e. cholinesterase inhibition, changes in neurotransmitter levels, behavioural effects) was made, in order to ensure that the neurotoxic NOELs retained would actually be the lowest. Moreover, it should be emphasized that among the 82 substances of the neurotoxicity database, 21 are organophosphate pesticides drawn from the Munro *et al*. (1996) database, for which the NOELs recorded were the lowest retrieved from the literature, that is, among all endpoints.

Immunotoxicity endpoint. In the evaluation of the immunotoxicity endpoint, the first screening for immunotoxic substances consisted in selecting substances among the 50 known or suspected immunotoxins from the Luster *et al*. (1992, 1993) database. Then, the criteria for immunotoxicity as established by Luster *et al*. (1992, 1993) was applied before any substance could be included in the database. On the one hand, such an approach has limited the immunotoxicity database to a relatively small number of substances, but on the other hand has allowed us to restrict this immunotoxicity endpoint evaluation to sole substances satisfying Luster's criteria for immunotoxicity, ensuring again that the database would be heavily weighted to demonstrated or highly suspected immunotoxins.

Developmental toxicity endpoint. In the evaluation of the developmental toxicity endpoint, a strong emphasis was put on selecting NOELs for manifestations of developmental effects other than terata: for instance behavioural dysfunction, growth alteration or embryo-foetal death, because such effects occur more frequently and at lower doses than gross morphological abnormalities (Ulbrich and Palmer, 1996; Vorhees, 1987). Therefore, it was expected that the selected NOELs would be the lowest and would give the worst case estimate of the 5^{th} centile NOELs and human exposure threshold. In addition to this special focus on possible low-dose-occurring developmental effects, there was also a careful selection regarding the design (i.e. two-, three- or multi-generation studies where exposure to test compounds began before mating, continued during mating, throughout gestation and lactation, until weaning), and the duration of the study (i.e. long-term studies), in order to assure that any stage of the development would be taken into consideration, and that effects would be detected at any time of the lifespan of the organism.

Considerations in comparing NOELs for various endpoints

Comparison of the NOEL or LOEL for qualitatively different hazards and endpoints needs to be conducted critically and in the light of the pivotal studies from which the NOELs/LOELs are derived. There are some caveats to the simplistic conclusion that the endpoint giving the lowest NOEL is indicative of a greater relevance to the establishment of an ADI for humans. These relate to different toxicokinetic or toxicodynamic considerations for the different endpoints.

As discussed by Renwick (1993) and adopted by the IPCS (1994), derivation of the ADI from a NOEL requires the application of appropriate safety or uncertainty factors to allow for inter-species and inter-individual differences in toxicokinetics or toxicodynamics. Although a default value of 100 for the composite uncertainty factor has commonly been adopted, this should be modified in the light of comparative data in the test species and humans. As a result, an endpoint with a lower NOEL may require the application of a lower uncertainty factor and hence a higher ADI may result.

A factor that needs to be considered in comparing NOELs for different toxic endpoints is that the dose and circumstances of exposure may affect the qualitative nature of the hazard. There is a tendency for some regulatory authorities to consider applying additional "safety" factors based on the nature of the hazard, most notably progressive or irreversible toxicity such as carcinogenicity or teratogenicity. However, the logic of this is questionable if there is a clear NOEL for the effect and if this effect is only seen at doses above the LOEL for some other manifestation of toxicity (e.g. above the MTD); the nature

of the toxicity at higher doses is irrelevant to doses at which there is no effect. Such ''overdose'' toxicity applied with exaggerated empirical (rather than data-derived) safety factors may lead to one endpoint dominating the safety evaluation process when the hazard relevant to the circumstances of human exposure in food may be different. It is not sensible to consider, for example, teratogenicity which occurs only at maternally toxic doses, or compromised immune function following inanition due to toxic anorexia or simply impalatability as a basis for calculating an ADI. However, such secondary effects may result in compounds being included in databases of teratogens or immunotoxicants.

It is not uncommon to find that there are qualitatively different toxic hazards at different doses or on different modes of exposure. This may arise from saturation of particular metabolic processes or from different concentration dependence; that is, slope of the concentration/rate curve, for different pathways of metabolism. Similarly, bolus doses by gavage may induce effects not elicited by the same daily dose spread over a day through diet either due to local irritant effects or ready absorption and saturation of detoxification mechanisms.

Toxicodynamic considerations may also indicate that dose- or concentration-dependent differences might occur between species so that the qualitative nature of the hazard may be more or less significant in man than test species. For example, reduced uncertainty factors may be required for some receptor-mediated toxic endpoints in rodents, such as those dependent on interaction with the peroxisome proliferator receptor (PPR). Indeed, some mechanisms of toxicity are considered species specific (e.g. $\alpha_{2\mu}$-globulin mediated renal toxicity/carcinogenicity in rats) and hence the toxicodynamics may be considered irrelevant for human safety evaluation purposes. In these circumstances, NOELs derived for these effects in rodents may be of no significance for human safety evaluation.

With regard to some of the endpoints under consideration in this document, the databases are somewhat limited and the methodology not generally validated for the purposes of safety evaluation of chemicals in food. Hence, substances may be classified as neurotoxic or behavioural toxins based on data from occupational exposure where the route of exposure, incurred dose and consequent toxicity may not readily be translated to determine a NOEL which is applicable to dietary exposure and may not cover the developmental phases of life which are important in the safety evaluation of chemicals in food.

From the foregoing, it is clear that simplistic comparisons of NOELs for different toxic hazards may need refining before reaching conclusions about whether the threshold of concern for a particular toxic endpoint is lower than that for genotoxic carcinogens. Notwithstanding the above, in the present analysis the lowest NOELs have been used as a basis of comparison, in order to be conservative. This may have led to an overestimate of the sensitivity of humans to a particular endpoint, and its relevance to that endpoint.

Data analysis: comparison of specific endpoints sensitivity with other forms of toxicity and derivation of human exposure thresholds

Neurotoxicity and developmental toxicity endpoints. In order to evaluate the sensitivity of various non-carcinogenic endpoints, the NOELs selected for the specific endpoints for which an adequate number of NOELs existed (i.e. neurotoxicity, developmental neurotoxicity and developmental toxicity) were plotted as a cumulative distribution. The cumulative distribution of NOELs of class III chemicals from the Munro *et al*. (1996) database also was plotted. In addition, the NOELs selected for the present specific endpoints evaluation were combined with the class III NOELs (duplicates were removed and the lowest values were retained) and plotted to determine whether any statistically significant changes in the distribution would occur.

Initially, a simplified, computerized version of the Kolmogorov–Smirnov test was conducted to determine whether there are any significant differences between the various cumulative distributions. The results of this analysis indicated that there were no statistically significant differences between the distributions, although the cumulative distribution for neurotoxicity reached borderline significance. To further ensure the accuracy of this analysis, the data were re-analysed using the same methodology described in the Munro *et al*. (1996) paper.

In order to compare the cancer risks with those from other endpoints, the distribution of 10^{-6} risks for carcinogens from the Gold *et al*. (1989) database calculated using the methodology of Krewski *et al*. (1990) was compared with the cumulative distributions of NOELs for non-cancer endpoints divided by a factor of 100. This value was chosen because this factor is conventionally used to derive an ADI for non-cancer endpoints.

Immunotoxicity endpoint. In the specific case of immunotoxic endpoint, although the immunotoxicity database contains 37 highly selected substances, only 15 NOELs were clearly defined, and it was felt that this number was too limited to plot a cumulative distribution of NOELs. Therefore, the approach chosen was that previously used by Munro and Kroes (1998) and Munro *et al*. (1999) in their evaluation of the immunotoxicity endpoint's sensitivity. NOELs for immunotoxic endpoints were compared with corresponding non-immunotoxic endpoints NOELS. For the substances lacking immunotoxic NOELs but having immunotoxic LOELs, the immunotoxic LOELs were compared with corresponding non-immunotoxic LOELs. Some substances had immunotoxic NOELs with corresponding non-immunotoxic LOELs, or immunotoxic LOELs with corresponding

non-immunotoxic NOELs. For these substances, NOELs were compared with LOELs divided by a conservative factor of 10 to adjust for differences between NOELs and LOELs (Dourson *et al*., 1996). Then, the immunotoxic NOELs or LOELs were divided by the corresponding non-immunotoxic NOELs or LOELs. The resulting ratios were recorded. Ratios of 1 and above correspond to substances for which the immunotoxic endpoint measured was as sensitive as, or less sensitive than other endpoints. Ratios below 1 correspond to substances for which the immunotoxic endpoint measured was the most sensitive endpoint retrieved from the literature review.

Results

Evaluation of specific endpoints: neurotoxicity and developmental neurotoxicity, immunotoxicity, developmental toxicity endpoints

The five cumulative distributions were plotted and are shown in Fig. 1.

Neurotoxicity and developmental toxicity endpoints. Statistical analysis indicated that only the cumulative distribution for neurotoxicity differed significantly from the other four distributions for non-cancer endpoints.

As is shown in Table 2, and in Fig. 2 where distributions of chemical carcinogens and non-cancer NOELs/100 were plotted in a semi-log form, there is an extremely large margin between the centiles of 10^{-6} for chemical carcinogens calculated based on the Gold Carcinogenic Potency Database (CPDB) and the centiles of various non-cancer NOELs/100. The data demonstrate that the distribution of 10^{-6} risk for carcinogens provides a much more conservative basis to derive a TTC than the distribution of NOELs for neurotoxicity or other endpoints divided by a factor of 100.

Immunotoxicity endpoint. Given the limited number of substances in the immunotoxicity database, a cumulative distribution could not be reliably established. Immune NOELs were therefore compared with other non-cancer endpoints NOELs for the same substances as described by Munro and Kroes (1998) and Munro *et al*. (1999).

10 out of the 37 substances from the immunotoxicity database had immune NOELs with corresponding non-immune NOELs. 19 of these substances had no immune NOELs but had immune LOELs with corresponding non-immune LOELs. Five additional substances had immune NOELs with corresponding non-immune LOELs, and three substances had immune LOELs with corresponding non-immune NOELs. Immune NOELs or LOELs were divided by the corresponding non-immune NOELs/LOELs. The frequency of the resulting ratios are presented in Fig. 3.

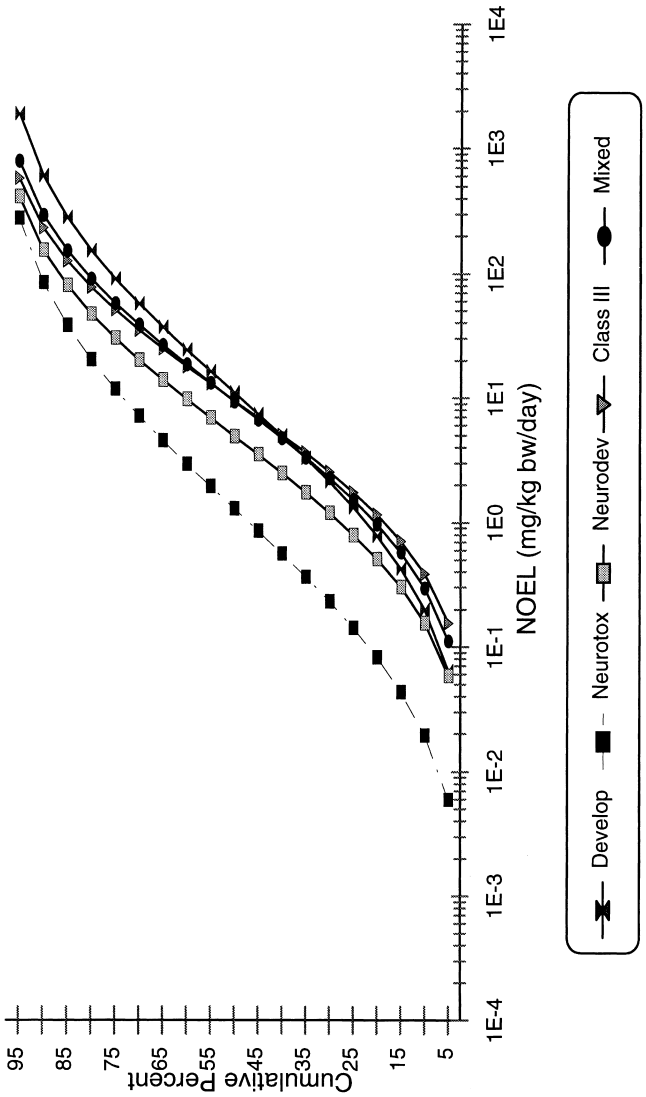
This comparison indicated that in 15 out of 37 cases, immunotoxicity was the most sensitive endpoint (ratio < 1). However, it should be noted that these results do not mean that immunotoxic NOELs are particularly low. Indeed, in general terms the distribution of immunotoxic NOELs for the group of immunotoxicants examined here did not appear to differ from the distribution of non-specific endpoints NOELs for the same compounds. Even in the most extreme case of this database, namely arsine, and by an irrelevant route, the lowest immunotoxic NOEL is in the range of 0.015 mg/kg body weight/day. This immune NOEL divided by a factor of 100 is equivalent to 9 μg/person/day which is higher than the threshold of toxicological concern of 1.5 μg/person/day.

Discussion

Analyses were conducted to determine whether there were any significant differences between the various distributions of NOELs for specific non-carcinogenic endpoints (those for which an adequate number of NOELs existed; that is, neurotoxicity, developmental neurotoxicity, developmental toxicity). The results show that there is no significant difference between the cumulative distributions for the neurodevelopmental and the developmental toxicity endpoints, and the cumulative distribution of NOELs from Class III chemicals from the Munro *et al*. (1996) database. In addition, NOELs from the specific endpoints evaluated here were combined with the class III NOELs and the resulting distribution was plotted. There was no significant difference either, between the latter distribution (identified as ''mixed'' in Fig. 1 and Table 2) and the distribution of class III NOELs.

The only cumulative distribution differing statistically from the other distributions was that for neurotoxic NOELs. It is not really surprising that this distribution appears more sensitive than the other distributions (i.e. endpoints) since the marker for neurotoxicity typically used to derive the NOELs in this dataset was cholinesterase inhibition, which is extremely sensitive. Furthermore, when the neurotoxicity dataset is added along with the other two datasets to the substances comprising structural class III of the Munro *et al*. (1996) database and the resulting distribution is plotted (as ''mixed'' in Fig. 1 and Table 2), no change is observed in the cumulative distribution between the class III dataset and the combined dataset. This indicates that, within the power of the analysis, these endpoints are comparable to other Class III compounds.

It is also important to note that most of the neurotoxic substances for which data were available have structural alerts and should be readily identified. As an illustration, a complementary investigation was undertaken on the database of Munro *et al*. (1996). All chemicals (over 600 substances) from this database were screened for NOELs falling below the 5th centile estimated for class III chemicals (substances with the highest toxic potential). The 5th centile estimated for this structural class is



" Mixed " = Combination of developmental, neurotoxic, neurodevelopmental, and Class III NOELs.

Fig. 1. Cumulative distributions of NOELs for non-cancer endpoints.

Table 2. Comparison of 10^{-6} risk for chemical carcinogens with centiles of various non-cancer NOELs/100

Data sources	No. of chemicals	Centile (ng/kg bw/day)				
		10th	25th	50th	75th	90th
CPDB (Rulis, 1986)	343	0.679	3.81	25.90	176	991
CPDB (Gold *et al.*, 1989)	492	0.401	2.48	18.70	142	876
Developmental [a] (present study)	81	1980	13,300	110,000	906,000	6,070,000
Neurotoxic [a] (present study)	45	195	1420	12,900	117,000	851,000
Neurodevelopmental [a] (present study)	30	1550	7960	49,000	301,000	1,550,000
Class III [a] (Munro *et al.*, 1996)	448	3841	17,510	94,420	509,300	2,321,000
Mixed [a, b]	501	2960	15,200	93,300	574,000	2,950,000

[a]Centile/100. [b]Combination of developmental, neurotoxic, neurodevelopmental and class III NOELs.

0.15 mg/kg body weight/day. The NOELs that fall below this value are presented in Table 3.

The substances in the neurotoxicity database for which neurotoxicity was the most sensitive endpoint are depicted in Table 4.

Inspection of Table 3 indicates that among substances that fall below the 5th centile for structural class III, the majority do not have neurotoxicity as the most sensitive endpoint. In every case except for dimethoate, neurotoxicity was not the most sensitive endpoint.

From Table 4, it is clear that among 20 substances for which neurotoxicity was the most sensitive endpoint, six substances had a neurotoxic NOEL falling below the 5th centile estimated for structural class III (0.15 mg/kg body weight/day).

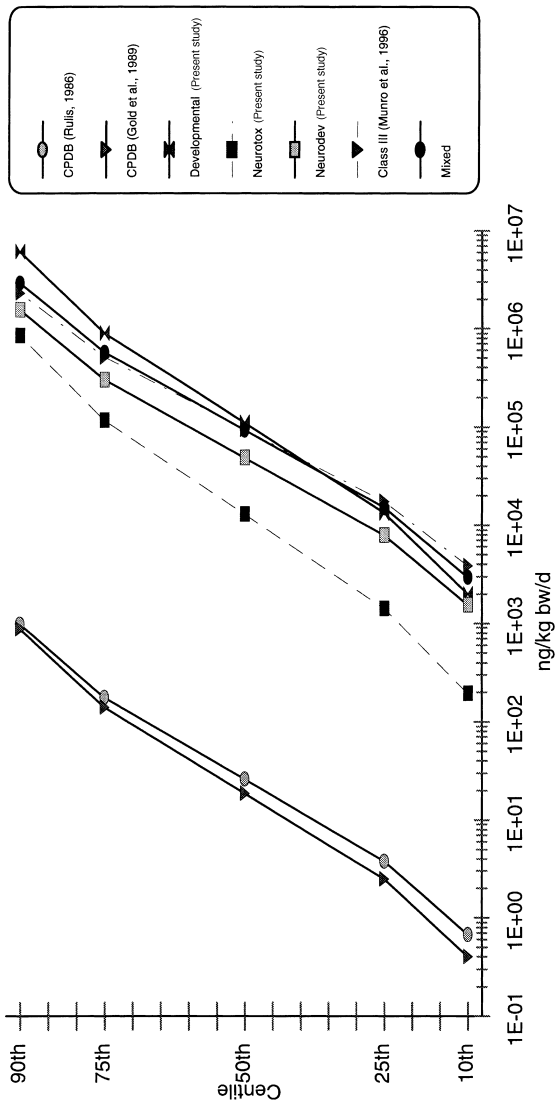
From observation of Tables 3 and 4, it appears clearly that neurotoxic substances exhibiting low NOELs (i.e. below the 5th centile estimated for Class III) are rare and correspond almost exclusively to pesticides. Similarly, when the specific neurotoxic endpoint is considered, it is apparent that only a small number of substances (identified as "§" in Table 4), have a neurotoxic NOEL lower than the 5th centile estimated for class III, most of them being organophosphate pesticides. Obviously, pesticides are specifically designed to be highly potent neurotoxins, and it is very likely that such substances would be readily identified for their structural alerts. Hence, the neurotoxicity database was heavily weighted to those substances with structural alerts and reported neurotoxic effects (e.g. 65 pesticides out of 82 substances in the neurotoxicity database). In addition, it should be remembered that the parameters selected were particularly sensitive as illustrated above (e.g. cholinesterase inhibition), and not necessarily associated with a functional deficit. For example, plasma cholinesterase was used in certain cases to determine a neurotoxic NOEL, which certainly represents a "worst-case scenario" since plasma cholinesterase inhibition is not necessarily associated with neurologic dysfunction. This emphasizes again that conservative assumptions were used at every step of the present analysis which embraces a "worst-case scenario". Finally, when going back to a general toxicity database where substances were not selected for specific toxicity endpoints [i.e. Munro *et al.* (1996) database], it appears clearly that neurotoxicity is not of particular concern [e.g. dimethoate is the only substance among over 600 of the Munro *et al.* (1996) database for which the NOEL falls below the 5th centile for class III and the toxic effect reported is neurotoxic—see Table 3].

Table 3. Most sensitive endpoints for substances falling below the 5th centile of structural class III

Substance	NOEL (mg/kg bw/day)	Endpoint measured
Isopropyl alcohol	0.018	teratogenesis
Avermectin B1	0.03	teratogenesis
Bidrin	0.1	reproductive
Chlordane	0.055	liver
Dieldrin	0.005	liver
Dimethoate	0.05	neurotoxic
Disulfoton	0.05	multiple
Fenamiphos	0.1	body weight changes
Haloxyfop-methyl	0.005	organ weight changes
Hexachlorobenzene	0.08	liver
Merphos	0.1	blood
Methyl parathion	0.025	organ weight changes
Patulin	0.04	body weight changes
Quinalphos	0.03	blood
Sodium fluoroacetate	0.05	multiple
Terbutryn	0.1	blood
Trenbolone acetate	0.044	reproductive
Trenbolone hydroxide, 17-α	0.04	blood
Zeranol	0.02	ovary

With respect to the immunotoxicity endpoint, the results of the comparison between 37 immune NOELs (or LOELs) and 37 non-immune NOELs (or LOELs) for the same compounds confirm the conclusions of Munro and Kroes (1998) and Munro *et al.* (1999) regarding the sensitivity of the immunotoxic endpoint; that is, it is not a more sensitive endpoint than other forms of toxicity. The criteria of immunotoxicity defined by Luster *et al.* (1992, 1993) were used in screening for substances to be included in the database. Therefore, the evaluation of the immunotoxicity endpoint was conducted on a specific database heavily weighted to identified immunotoxic substances or compounds reported to cause lymphoid organ weight changes and/or haematological changes (Dean and Murray, 1990; Lu, 1990; Luster *et al.*, 1992, 1993).

Among these 37 candidate chemicals with the highest potential to exert effects on the immune system, 15 substances exhibited immune NOELs lower



" Mixed " = Combination of developmental, neurotoxic, neurodevelopmental, and class III NOELs.

Fig. 2. Distribution of chemical carcinogens and non-cancer NOELs/100.

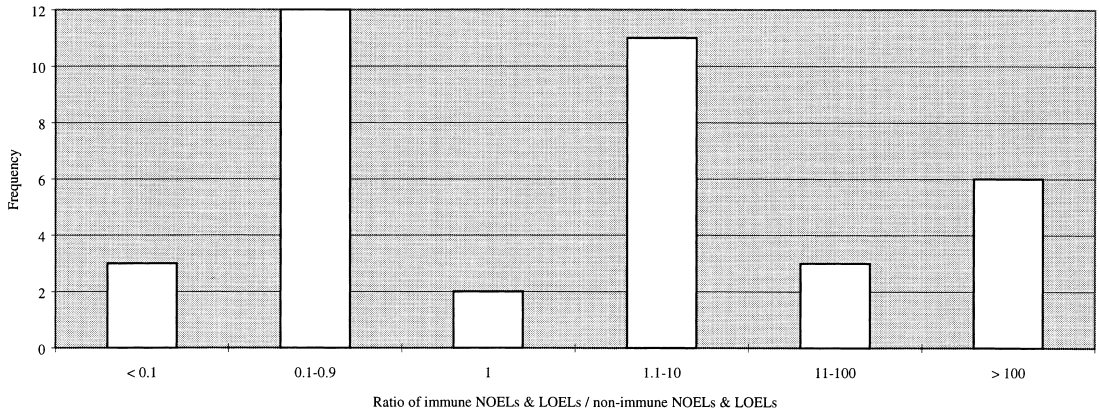


Fig. 3. Comparison of the sensitivity of the immunotoxicity endpoint *vs* general toxicity endpoints. The case of a selection of 37 well-characterized immunotoxicants.

than non-immune NOELs (for which the ratio calculated is below 1). It is very likely that the frequency of immunotoxicants in randomly selected substances (i.e. "the world of chemicals") would be significantly lower, and it is therefore considered that such results correspond, again, to a "worst-case scenario". Furthermore, it should be emphasized that the significance of the observed changes is uncertain when considering that the immune system has a reserve capacity and that mild, transient fluctuations in activity are unlikely to be of clinical significance. Also, the degree of alteration of the overall functional capacity has not been correlated yet to isolated immunological parameters as measured by the tests of immune function (Trizio *et al.*, 1988), and because of the complexity of the immune system and the variability of the immune status between individuals and groups, the changes recorded in such immune tests do not necessarily indicate that the immune system has been compromised.

Finally, as the specific immunotoxicity endpoint is shown not to be more sensitive than the other toxic endpoints generally tested, it can be concluded that potential immune effects would probably not occur at lower doses than general toxicity effects, and may not be given a greater concern when considering to establish a TTC.

Both Table 2 and Fig. 2 demonstrate that a very large margin exists between the distribution of thresholds derived from non-cancer endpoints and those derived from cancer endpoints. It should be noted that the lowest human exposure threshold of 88 μg/day for structural class III is 60 times higher than the 1.5 μg/person/day derived from cancer endpoints. Clearly, the thresholds derived from the carcinogenic potency database (i.e. TD_{50}s) are lower than thresholds derived from non-cancer endpoints, including neurotoxicity. It is important to note that the use of probabilistic methodology in deriving thresholds from TD_{50}s provides very conservative estimates for several reasons:

- The TD_{50}s in the carcinogenic potency database are derived from studies with chemicals tested in animals over a lifetime at the maximum tolerated

Table 4. Neurotoxic NOELs for substances for which neurotoxicity was the most sensitive endpoint

Substance	NOEL (mg/kg bw/day) §substance having neurotoxic NOEL < 5th centile estimated for Class III	Endpoint measured
Carbofuran	0.83	neurobehavioural
Cismethrin	2	neurobehavioural
Dichlorvos	0.23	inhibition ChE
Dimethoate	0.05§	inhibition brain, RBC, plasma ChE
Ethephon	15	inhibition RBC, plasma ChE
Ethion	0.20	inhibition plasma ChE
Ethyl-*p*-nitrophenylphenylphosphorothioate	0.01§	delayed neurotoxicity
Fonophos	0.50	inhibition RBC, plasma ChE
Lindane	0.83	neurobehavioural
Malathion	5	inhibition brain ChE
Mancozeb	2.73	neurobehavioural
Merphos	0.10§	inhibition RBC ChE
Methamidophos	0.10§	delayed neurotoxicity
Pirimiphos-methyl	0.50	inhibition plasma ChE
Pydrin	0.83	neurobehavioural
Quinalphos	0.03§	inhibition plasma ChE
Tetraethyldithiopyrophosphate	0.50	inhibition RBC, plasma ChE
Acrylamide	0.06§	neurohistological

dose (MTD), unlike typical human exposure conditions.

- The procedures used by Gold *et al.* (1984, 1989) in developing the TD_{50}s contained many conservative assumptions.
- Linear extrapolation from the lowest TD_{50} for each substance was used in the calculation of the distribution of the 10^{-6} risks and, hence, the threshold value of 1.5 μg/person/day.

In conclusion, the present analysis included compounds with structural alerts for genotoxicity and carcinogenicity. Keeping in mind that the analysis demonstrated that the addition of several non-carcinogenic substances to structural class III did not alter the distribution of NOELs for this class, it may be possible to accept a higher threshold (e.g. the TTC for chemical substances from class III), if structural alerts for genotoxicity and carcinogenicity can be ruled out.

Special case: endocrine toxicity endpoint evaluation

Endocrine active compounds and adverse health effects

There is growing concern about possible harmful consequences of exposure to xenobiotic compounds that are capable of modulating or disrupting the endocrine system. This concern for endocrine-disrupting chemicals is directed at both wildlife and humans (CSTEE, 1999; EC, 1996). Substances with oestrogenic potential are the principal concern, but other steroid hormonal effects are also receiving increasing attention. Effects from exposure to these compounds have been observed in a variety of fish and wildlife species, especially during developmental stages (EC, 1996). Adverse effects from exposure to such chemicals have been reported in endocrine and reproductive systems in laboratory rodents (Janssen *et al.*, 1997). It has been suggested that anthropogenic chemicals characterized as ''endocrine modulators'' might be associated with breast cancer, and could potentially alter the reproductive capability of humans (Rudel, 1997). However, although there are associations between endocrine-disrupting chemicals, so far investigated, and human health disturbances, a causative role of these chemicals in diseases and abnormalities possibly related to an endocrine disturbance has not been identified (CSTEE, 1999; DFG, 1998).

There is currently no reliably documented evidence of effects from oestrogenic substances in the human food supply (CSTEE, 1999; DFG, 1998). The role of such chemicals in human health is currently the subject of intensive research to determine the significance of exposures to environmental endocrine modulators, in the absence of critical knowledge on effects, exposure or dose–response relationships (Rudel, 1997). It is imperative that it be determined whether endocrine effects reported in wildlife species and/or responses seen in *in vitro* assays are likely to be applicable to humans. In this context, it is useful to examine the physiology of the human endocrine system and the respective roles of endogenous endocrine modulators in the maintenance of hormonal balance. Because of the attention directed towards oestrogenic modulators, the focus of this section will be on this particular class of agents.

The aim of this section is to evaluate the possible adverse effects in humans of environmental substances with oestrogenic activity, in order to distinguish them from endogenous hormones and pharmaceuticals used for therapeutic purposes.

Human endocrine system, hormones and their roles

The endocrine system is one of the most complex and integrated systems of the human body. It controls and modulates many bodily functions at all stages of an individual's development. The endocrine system, along with the nervous system, supports and regulates homeostatic functions of the body. This function of the endocrine system is achieved through secretion of chemical messengers called hormones that provide subtle but powerful instruction to specific cell types or organs (Eubanks, 1997).

Hormones are of diverse structural classes composed of glycoproteins, polypeptides, steroids, modified amino acids, catecholamines, prostaglandins and retinoic acid. They circulate freely in the blood at very low concentrations (i.e. 10^{-9} or 10^{-12} g/ml) or may be found bound to proteins. To induce endocrine responses, hormones typically bind to specific receptors (EPA, 1997).

Natural substances such as oestrogens, androgens, hormones active on thyroid and pituitary glands, peptide or neuroendocrines are potent bioactive compounds that participate in the maintenance of homeostasis. This state of balance is maintained by a number of central nervous system-pituitary-target organ feedback pathways that regulate various bodily functions. In this manner, a specific hormone may affect several target tissues and systems, including bone, skin, reproductive, cardiovascular and possibly central nervous and immune systems (Neubert, 1997).

Interference with hormonal signals may have significant biological consequences. Two examples of intentional modulation of endocrine function are the female birth control pill, or the inhibition of 5α-reductase to slow the growth of the ageing males' prostate (Chapin *et al.*, 1996). The current public and scientific concern is focused on possible unintentional modulations of natural endocrine function by ''environmental estrogens'' displaying the ability to mimic endogenous oestrogenic compounds and potentially induce adverse effects on human health (i.e. breast cancer) and reproductive capability (i.e. decrease in sperm count and fertility) (Colborn, 1995; Kavlock *et al.*, 1996).

Natural sex hormones play critical roles in the development and maintenance of male and female sexual functions, including sexual differentiation and reproduction (i.e. development, puberty, behaviour, gametogenesis and integrated sexual function) (Kavlock *et al.*, 1996; Neubert, 1997). 17β-oestradiol plays an important role in regulating the growth and maturation of reproductive tissues including the vagina, ovary, oviduct, mammary glands, with the main target organ being the uterus. Other activities of oestrogens include direct influences on the pituitary, hypothalamus, and specific brain regions. Less direct effects include the regulation of growth and metabolism in various tissues such as bone, liver and cardiovascular system (Miller, 1996).

In humans, the naturally occurring oestrogens include 17β-oestradiol, oestrone and oestriol. Oestradiol is the most potent natural oestrogen targeting the reproductive tract, bone synthesis, and effecting fat distribution throughout the body. The primary actions of estradiol at the cellular level comprise induction of cell growth, and modulation of the effects of other hormones (Janssen *et al.*, 1997). Oestrone (mainly in the sulfate form) is found predominantly in the male, and oestriol (and its glucuronide forms) is largely secreted during pregnancy by the foetal–placental unit (Miller, 1996).

Natural fluctuations in hormone levels

Physiological regulation of hormone levels. In the human endocrine system, the control of hormone production is regulated through a complex negative-feedback pathway that responds to fluctuations of circulating hormone concentration. The synthesis and secretion of endocrine substances is modulated through levels of circulating bound and unbound hormones (Miller, 1996).

Typically, concentrations of hormones within the body are low, but the highly responsive feedback mechanisms provide extremely subtle and adaptable control of hormone levels. Some physiological states (e.g. pregnancy and menopause) or environmental challenges may modulate hormone concentrations as well as receptor activities. Generally, as a peak of hormone production occurs, there is a natural inhibition of the primary synthetic pathway with a subsequent down-regulation of the substance (EPA, 1997). The physiologic state and the concentration of unbound hormone influences the number of unoccupied specific receptors on target cells. This phenomenon is termed homologous "desensitization" or "down regulation". It is this response that has been one focus of concern since substances capable of acting as hormone mimics may alter natural hormone production and balance. Substances present in the environment capable of modulating endocrine function could affect an imbalance in the delicate homeostatic equilibrium (EPA, 1997).

Natural fluctuations of oestrogen levels within the bounds of homeostasis

Examples of rapid fluctuations in plasma levels of oestradiol have been documented in normal premenopausal women, suggesting an episodic secretion of the hormone (Reed, 1983). Landgren *et al.* (1980) have also measured plasma levels of oestradiol and other hormones in healthy women with a history of regular menstrual cycles and reported fluctuations of plasma oestradiol levels between 41 and 101 pg/ml (during the first 6 days of the cycle) and more than 188 pg/ml for the pre-ovulatory peak (see Tables 5 and 6).

Concentrations of hormones within the female also fluctuate during special physiologic periods such as pregnancy. For example, plasma concentrations of oestradiol, oestrone or oestriol are known to increase from early to late pregnancy. Changes have been associated with circadian rhythm showing fluctuations in plasma levels of unconjugated oestriol during late pregnancy (Goebel and Kuss, 1974; Katagiri *et al.*, 1976; Reck *et al.*, 1979; Unnérus, 1979), and even sub-hourly variations in levels of unconjugated oestriol and oestradiol were reported by Buster *et al.* (1978).

Plasma concentrations of oestradiol are also present in healthy males and have been measured by Lenton *et al.* (1978).

Among individuals, there is a wide range of natural variation in homeostatic plasma levels of oestrone and oestradiol. These may vary considerably in both women and men (from two to five times higher than the physiologic levels), depending on several factors such as weight, age or diet, without causing any specific pathology (Reed, 1983; cf. Tables 5 and 6).

Natural hormone levels commonly fluctuate monthly, daily and even hourly in healthy individuals. Without evidence of specific adverse effects, suggesting that even though concentrations of hormones vary within the body, the changes are absorbed or modulated by adaptable control mechanisms, so that ultimately, homeostasis is maintained (Reed, 1983).

Environmental endocrine modulators

Interference of endocrine active environmental compounds in the mechanisms of hormonal responses. A number of exogenous substances have been identified as having the potential to act as modulators of normal hormonal responses. These so-called "endocrine disrupters" or "endocrine modulators" may be natural products or synthetic chemicals capable of direct interaction with oestrogen receptors, other hormones, or transcription factors in the biochemical pathway of hormone activity. These environmental oestrogens can enhance (an agonist), or inhibit (an antagonist) the action of endogenous hormones. In some instances, this group of substances can act as both an agonist and an antagon-

Table 5. Plasma concentrations of sex hormones (from Reed, 1983)

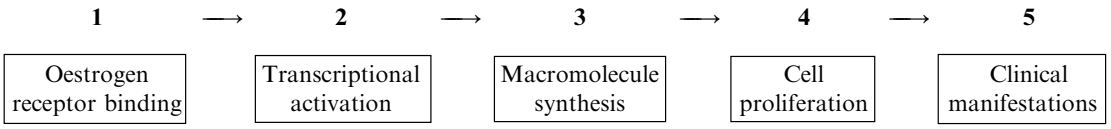
Premenopausal women[1] (relative to LH peak)	−8 days	mid peak	at menstruation
Estradiol n = 68 normal women	275 pmol/litre	1030 ± 110 pmol/litre	225 pmol/litre
90% population 90% probability	135 to 585 pmol/litre	400 to 2400 pmol/litre	90 to 475 pmol/litre
Postmenopausal women[2]	With breast cancer n = 71	Without breast cancer n = 166	
Estradiol	66.7 ± 4.4 pmol/litre	48.6 ± 4.0 pmolitre	
Estrone	127.5 ± 6.6 pmol/litre	119.8 ± 5.2 pmol/litre	
Estrone sulfate	610.4 ± 82.6 pmol/litre	647.7 ± 28.8 pmol/litre	
Testosterone	0.75 ± 0.7 nmol/litre	0.55 ± 0.04 nmol/litre	
Androstenedione	8.52 ± 1.6 nmol/litre	2.97 ± 1.5 nmol/litre	
Male (normal)			
Estradiol (fluctuates with weight)	80 ± 17 pmol/litre		
Estrone (85% of estrone in plasma is an estrone sulfate)	92 ± 22 pmol/litre		

[1]From Reed M.J. (1983) The oestrogens. In *Hormones in Blood*, 3rd edn, Vol. 4, ed. A.H. Gray and V.H.T. James. Academic Press, New York. [2]From Dorgan *et al.* (1997) Serum sex hormone levels are related to breast cancer risk in post menopausal women. *Environmental Health Perspectives* **105** (Suppl. 3).

ist, depending on the target tissue (i.e. tamoxifen) (EPA, 1997).

Oestrogenic effects can be induced by a variety of mechanisms, but they most commonly occur through binding of endocrine active compounds to nuclear receptors. Two different receptors (ERα and ERβ) have been identified up to now (Kuiper *et al.*, 1997). They show specific tissue distribution (Saunders *et al.*, 1997). Many agents have been identified that can bind to oestrogen receptors and act with varying degrees of efficacy as substitutes for endogenous oestrogens. They include natural products such as coumestrol, genistein and zearalenol, and some drugs such as diethylstilboestrol, ethinylestradiol and tamoxifen. Industrial chemicals such as pesticides (i.e. *o,p′*-DDT, dieldrin) and alkylphenols (i.e. bisphenol A, *p*-nonylphenol, etc.) have also been reported to be oestrogenic (EPA, 1997; Ramamoorthy *et al.*, 1997; Ziegler, 1997). Interference with oestrogen receptor binding can be measured, while other effects involving metabolic inhibition or induction (affecting steroidogenesis, enzymes that modify hormones, plasma transport proteins, and neurotransmitter levels) are difficult to establish. Examples of endocrine effects induced by changes in steroid metabolizing enzymes include decreased levels of androgens (i.e. testosterone) following exposure to dioxins (Moore *et al.*, 1991) or ketoconazole (Feldman, 1986; Morita *et al.*, 1990; Vanden Bossche, 1985). Certain oestrogenic compounds can also inhibit specific enzymatic steps in the biosynthetic pathway of steroidogenesis, or inhibit oestrogen biosynthesis due to exposure to aromatase inhibitors (i.e. the fungicide fenarimol).

Many hormonal responses are initiated by ligand binding to receptors, followed by transcriptional activation of responsive genes. For oestrogenic responses, some authors have described the key-steps of the process (Calabrese *et al.*, 1997):



Multi-step process of oestrogenic functions

Table 6. 17β-Estradiol plasma concentration ranges (from Reed, 1983)

	Plasma concentration		Total in 6 litres blood
Male:	37–130 pmol/litre	10–35 pg/ml	60–210 μg
Female:			
Follicular phase day 0–6	110–440 pmol/litre	30–120 pg/ml	180–720 μg
Luteal phase days 7–13 and 15–21	370–770 pmol/litre	101–210 pg/ml	606-1260 μg
Preovulation: day 14	550–1290 pmol/litre	150–351 pg/ml	900–2106 μg
Postovulation: day 22–28	37–130 pmol/litre	10–35 pg/ml	60–210 μg
Postmenopausal	48.6 ± 4.0 pmol/litre	13 ± 1 pg/ml	78 ± 6 μg

The process described above is sensitive at several points to the disruption of steps in: receptor binding, transcriptional activation or cell proliferation. The effects of an endocrine disrupter may be reversible or irreversible, immediate or latent. Dose, body burden, timing and duration of exposure at critical periods of life are important factors to be examined for assessing these potential adverse effects (EPA, 1997).

Endocrine active environmental compounds. An endocrine disrupting chemical (EDC) is defined as an exogenous agent that has the potential to interfere with the synthesis, secretion, transport, binding, action, or elimination of endogenous hormones responsible for the maintenance of homeostasis (EPA, 1997). These environmental compounds with endocrine activity can be categorized according to their origin (Janssen *et al.*, 1997):

- Synthetic hormones used in medicine and veterinary medicine.
- Environmental chemicals with hormone-like activity.
- Plant constituents with hormone-like activity (''phytoestrogens'') and substances produced by fungi (''mycoestrogens'').

Although humans are widely exposed to naturally occurring estrogenic substances in the diet (e.g. isoflavones, lignans, coumestanes, indo-3-carbinoles, or mycotoxins such as zearalenone) (DFG, 1998; Kuhnau, 1976; Verdeal and Ryan, 1979), the concern was originally raised with regard to anthropogenic environmental pollutants (e.g. *p,p′*-methoxychlor, PCBs metabolites, *o,p*-DDT, pesticides such as endosulfan, toxaphene, dieldrin or alkylphenols such as bisphenol A) (Safe, 1995).

During the past few decades, substances from various chemical classes have indeed been identified that exhibit the capacity to modulate certain functions of sex hormones. Such a qualitative finding is of great scientific interest, but may have little significance for relevant human exposures. Indeed, as noted before, a causative role of these chemicals in diseases and abnormalities possibly related to an endocrine disturbance has not been identified, and adverse health effects associated to these chemicals remain highly controversial with no consensus reached yet (Colborn, 1995; CSTEE, 1999; EC, 1996; Safe, 1995; Sharpe and Skakkebaek, 1993; Wolff *et al.*, 1993).

As any toxicological evaluation of environmental endocrine modulators should address qualitative as well as quantitative aspects, a quantitative risk assessment for humans is needed. This should take into account the extent of possible human exposure and the biological potency of the chemical substance (Neubert, 1997). Therefore, the next sections will address three specific questions:

1. What are the human exposures to synthetic environmental oestrogenic chemicals?
2. Are the biological potencies of environmental chemical oestrogens and phytoestrogens comparable to that of endogenous hormones?
3. In view of the cumulative exposures to environmental chemical oestrogens and phytoestrogens, and taking into account the actual oestrogenic potencies of these substances, does the risk to human health appear to be significant?

Significance of human exposures to oestrogenic chemicals

Without commenting on the precision of his estimates, Winter (1992) calculated that the exposure to oestrogenic active compounds based on the global dietary intake of oestrogenic pesticides was approximately 2.5 μg/person/day. Exposures for different age groups from 6–11 months, 14–16 years to 60–65 years old were estimated at a total of 0.1063 μg/kg body weight/day, 0.0416 μg/kg body weight/day and 0.033 μg/kg body weight/day of the oestrogenic pesticides DDT, dieldrin, endosulfan and *p-p′*-methoxychlor (Winter, 1992).

The dietary intake of flavonoids from foods was estimated to constitute an average daily intake ranging from 1020 to 1070 mg/person/day (winter and summer season, respectively) (Safe, 1995). Taking into account the oestrogenic potencies of pesticides, which are probably lower by two orders of magnitude than that of flavonoids, three points are worth noting:

First, the 2.5 μg/person/day estimated level of exposure to oestrogenic pesticides equates to 0.0002% of the overall exposure to oestrogenic compounds from the diet. That is, exposure to oestrogenic chemicals (i.e. pesticides) counts for a negligible part of the total oestrogenic burden from the diet, in comparison with the major intake of naturally occurring bioflavonoids. Moreover, since these levels were measured in foodstuffs and not necessarily in prepared food, it is likely that this is an overestimate.

Secondly, the doses of methoxychlor (20 μg/kg body weight/day) and *o,p′*-DDT (20 μg/kg body weight/day) necessary to alter urine-marking behaviour in the male offspring of treated pregnant mice (vom Saal *et al.*, 1995) may be considered minimal doses for behavioural modification in rodents. If this is a sensitive marker of behavioural modification induced by endocrine disrupting chemicals, then it is likely that effects in humans, resulting from exposure levels about 2.5 μg/person/day which are 500 times lower than the minimal effective dose of methoxychlor and *o,p′*-DDT administered to rodents (vom Saal *et al.*, 1995), would be insignificant.

Thirdly, O'Connor *et al.* (1996) have demonstrated that known oestrogenic substances show thresholds of effects for several endpoints: oestriol was reported to modify uterine fluid imbibition at

50 μg/kg body weight/day, and tamoxifen was reported to alter serum prolactin levels at 500 μg/kg body weight/day, uterine stromal cell proliferation at 50 μg/kg body weight/day, and uterine weight at 50 μg/kg body weight/day. Compared to these dose levels, it is unlikely that a 2.5 μg/person/day exposure level would cause any effect, even if the potency were similar, which actually is not the case.

Potencies of environmental oestrogens relative to that of endogenous hormones

A variety of *in vitro* assays have been applied for characterizing the oestrogenic potential of chemicals (EPA, 1998). Some are based on cells transfected with one of the oestrogen receptors and reporter genes (e.g. yeast systems, human 293 kidney epithelial cell line) while others use oestrogen-responsive cell lines (e.g. human breast cancer cells MCF-7). Relative potencies for several oestrogenic substances can be estimated by comparing activity with an assigned standard [17β-estradiol (E_2) or diethylstilboestrol (DES)].

This approach has been explored by Soto *et al.* (1994, 1995) using MCF-7 cells. Briefly, effectiveness of environmental oestrogens has been quantitatively compared through the concepts of relative proliferative potency (RPP) and relative proliferative effect (RPE). RPP is defined as the ratio between the minimal concentration of oestradiol needed for maximal cell yield at 6 days and the dose of the test compound to achieve a comparable effect. RPE is defined as 100 times the ratio of the highest cell yield obtained with the chemical and with oestradiol. RPE gives an indication of whether the test compound is a full or partial agonist (Soto *et al.*, 1994, 1995).

By applying this approach, pesticides such as dieldrin, endosulfan, toxaphene, DDT and chlordecone were assigned oestrogenic potencies which were six orders of magnitude lower (0.000001) than that of oestradiol (Soto *et al.*, 1994, 1995). Similarly, further studies addressing naturally occurring oestrogenic substances such as bioflavonoids revealed oestrogen potency factors ranging from 0.001 to 0.0001 (Mäkelä *et al.*, 1994; Miksicek, 1993).

It is important to note that the relative potencies mentioned above should not be used as absolute values but should be considered as indicative. It is documented that the oestrogen receptor-mediated responses *in vitro* may vary significantly according to the test system (Odum *et al.*, 1998). Furthermore, most of the data have been generated with cell systems containing mainly ERα. Although preliminary results using cells transfected with ERβ confirms the low oestrogenic potency of natural and synthetic oestrogens (Barkhem *et al.*, 1998; Kuiper *et al.*, 1997), less information is presently available for ERβ-mediated response as compared with the ERα response.

As relative oestrogenic potencies of environmental chemicals are derived from *in vitro* studies, some authors have suggested that they could be even lower in *in vivo* models (Safe, 1995). However, based on rat studies, others have reported a less dramatic difference (1000- to 5000-fold) between potencies of chlordecone and oestradiol (Hammond *et al.*, 1979), suggesting that pesticides may persist and bioaccumulate, whereas opestradiol is rapidly metabolized (Soto *et al.*, 1994).

In summary, independent of the experimental system used, oestrogenic potencies calculated for anthropogenic oestrogens (e.g. pesticides, packaging materials) and bioflavonoids are several orders of magnitude lower than that of endogenous oestrogens. Therefore, biological consequences, if any, of exposure to environmental substances whose oestrogenic potency is about 0.001 to 0.000001 relatively to oestradiol, can most likely not be compared with endocrine responses induced by human natural hormones.

Impact of exposure to environmental oestrogens

As proposed by Safe (1995), by combining the estimated dietary exposure levels (from Winter, 1992) and the potencies of environmental oestrogenic pesticides (from Soto *et al.*, 1994, 1995) and bioflavonoids (from Mäkelä *et al.*, 1995; Miksicek, 1993) calculated relatively to oestradiol, it is possible to assess the impact of the intake of chemical- and phytoestrogens in Estrogen Equivalents (EE). As discussed above, the following calculation does not provide an absolute picture but should be considered as indicative.

- Estimated exposure to pesticides = 2.5 μg/person/day.
- Estimated exposure to bioflavonoids = 1070 mg/person/day.
- Relative potency of pesticides = 0.000001.
- Relative potency of bioflavonoids = 0.0001.

Estimated daily intake of EE (bioflavonoids in food + oestrogenic pesticides from foodstuffs):

$$1070\ \text{mg/person/day} \times 0.0001$$
$$+2.5\ \mu\text{g/person/day} \times 0.000001$$
$$= 107\ \mu\text{g/person/day (in EE)}$$

Amounts of EE from exposure to different sources are compared and presented in Table 7.

Isoflavones contribute about one-thirtieth (1/30) of the pharmaceutical dose used daily in post-menopausal therapy, while organochlorine substances contribute an insignificant amount to the total oestrogen load routinely encountered daily.

Discussion

Methodological issues. Bioassays measure the potency of a hormonal substance by quantifying a biological effect produced by this substance. In bioassays, the bioactivity of the test substance is

Table 7. (modified from Kavlock *et al.*, 1996)

Estimated mass balance of human exposures to environmental and dietary oestrogens

Sources

Oestrogens	Oestrogen equivalents (μg/day)
Morning-after pill	333,500
Birth control pill	16,675
Post-menopausal therapy	3350
Isoflavones in food	107
Organochlorine substances (environmental)	0.0000025

compared with that of a reference preparation. The *in vivo* bioassays investigate the biological potency of a hormone following administration to animals and quantification of a specific response. The *in vitro* bioassays are based on the biological effects produced by the hormonal substance when added to an *in vitro* preparation of the target tissue. Reliance on *in vitro* assays for predicting *in vivo* endocrine disrupter effects may generate false negative as well as false positive results, and these approaches are generally limited in their applications as screens. Therefore, results of numerous *in vitro* investigations suggesting an oestrogenic potential of those chemicals cannot be used for risk assessment without further validation. In particular, assessment of affinity to receptor preparations alone does not permit to draw conclusions regarding endocrine efficacy (DFG, 1998). Indeed, isolated systems are useful for studying mechanisms of action or structure–activity relationships, but can only provide a preliminary screen for one step of the process, while endocrine responses involve all the steps (Stevens *et al.*, 1997). Moreover, before exerting any deleterious effect, a toxicant must be transported to the target organ at concentration high enough to reach a critical dose. Thus, *in vitro* assays for oestrogenic activity often measure discrete biological responses at cellular level. They do not take into account the absorption, disposition, metabolism, excretion, bioaccumulation, and repair processes in the intact organism, which play a crucial role in the actual toxic response of a chemical (Reel *et al.*, 1997). Emphasis should rather be put on *in vivo* assays (CSTEE, 1999; DFG, 1998; EC, 1996). However, even the existing *in vivo* protocols for evaluating endocrine toxicity may be insensitive to subtle, low-dose effects. For instance, multiple agents may bind to a common site, acting as agonists, partial agonists or antagonists, so that exposure to endocrine active compounds leads to complex interactions between endogenous and exogenous agents, particularly at low doses (Markaverich *et al.*, 1978). Furthermore, no specific guidelines exist yet, that could help estimate dose–response relationships for hormonal effects (Rudel, 1997).

To address these issues and other concerns regarding the presence of endocrine disrupters in food, water, or other environmental media, and the potential risks they may pose to humans and wildlife, the US EPA was required to *"...develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect..."*. In 1996, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) was formed by EPA, to provide advice on how to design a screening and testing program for endocrine-disrupting chemicals. The Committee determined that a tiered approach would be most effective in utilizing reasonably available resources to detect endocrine-disrupting chemicals and quantify their effects. This approach is described in detail in the EDSTAC Final Report (EPA, 1998), but its core elements can be summarized as follows: (1) Initial sorting; (2) Priority setting; (3) Tier 1 screening to detect chemicals and mixtures capable of interaction with hormonal systems; (4) Tier 2 screening to determine whether a chemical or mixture exhibits endocrine-mediated adverse effects and to identify, characterize, and quantify those effects.

The EDSTAC also recognized that questions have been raised as to the adequacy of conventional toxicology study designs for assessment of endocrine active substances, particularly with regard to low-dose selection and the identification of no-observed-adverse-effect levels (NOAEL). To address these questions, the EDSTAC recommended that a project be performed to resolve the underlying uncertainties and controversy about these issues. The purpose of the project would be to address the nature of the dose–response curves for exogenous oestrogenic substances, in order to allow more informed judgement about appropriate toxicology study designs for substances having hormonal activity (EPA, 1998).

Potential for adverse endocrine effects in humans from exposure to environmental chemicals. The significance of possible hormonal changes induced by human exposure to environmental chemicals is questionable (CSTEE, 1999; EC, 1996). For example, in the oestrogenic effect process described by Calabrese *et al.* (1997), the probability of achieving the end result, that is endocrine alteration in an organism, is not equivalent at every step of the process. Effects observed during the early stages (i.e. receptor binding, transcriptional activation) have minor probability to produce actual hormonal disruption. As explained before, hormonal alteration in the human homeostatic system has not yet been correlated to the isolated effects measured in available endocrine tests. Based on the current knowledge, it is not likely that exposure to environmental oestrogenic chemicals at currently estimated intakes from food will be associated with any oestrogen-mediated adverse health effects. So long as the homeostasis is maintained by a wide range of equilibrium control mechanisms, deleterious effects are

expected to occur only when such mechanisms are overwhelmed (EPA, 1997).

Human exposures to environmental oestrogens and comparison with the 1.5 μg/person/day proposed threshold value. Although concerns and suspicion concentrate on oestrogenic chemicals present in the environment, it is felt that these may have little effects on endocrine function at dietary relevant doses (Shelby *et al*., 1996). The so-called phytoestrogens that occur in plants are quantitatively the most important hormonally active agents. In addition to these, many anthropogenic chemicals can also show oestrogenic potential. The latter may occur in food as a result of contamination, for example from the environment or from contact with plastic materials (DFG, 1998). As calculated earlier, an estimated dietary intake of pesticides with oestrogenic potential of 2.5 μg/person/day was compared with the average daily intake of all flavonoids in foods ranging between 1020 and 1070 mg/person/day. This demonstrated that exposure to anthropogenic oestrogenic chemicals is very low in comparison with exposure to naturally occurring phytoestrogens, and accounts for a negligible part of the total oestrogen burden from the diet (Table 7) (Kavlock *et al*., 1996; Winter, 1992). Furthermore, as far as the oestrogenic potencies are concerned, scientific data obtained so far suggest that oestrogenic compounds of anthropogenic origin, in comparison with endogenous hormones, possess only little hormonal activity like phytoestrogens (DFG, 1998).

In summary, results of animal studies do not suggest that hormonal effects are to be expected from the exposure to environmental oestrogenic chemicals found in foods. According to the present state of knowledge, the very low concentrations of such agents in foods would not present a health risk (DFG, 1998). The exposure threshold value of 1.5 μg/person/day would be sufficiently low to provide an adequate margin of safety against any adverse endocrine effect associated with dietary environmental substances.

The present evaluation focused on oestrogenic-disrupting chemicals because of the current public interest for concerns to public health. However, there are other classes of disrupters of endocrine systems. But for most of the thyroid disrupters, effects are usually observed at higher dose ranges; that is, milligrams instead of micrograms (Biegel *et al*., 1995; Fullerton *et al*., 1987; Lankas *et al*., 1995; NTP, 1989), and may therefore be of lesser concern regarding environmental and dietary exposures.

Special case: allergenicity

Food allergy and other adverse reactions to food

Adverse reactions to food. Adverse reactions to food include several different reactions that cause a wide range of symptoms by a variety of distinct mechanisms. The term "food allergy" is sometimes applied incorrectly to certain of these reactions. Figure 4 presents and clarifies the different causes and mechanisms involved in food allergy and other adverse reactions to foods.

Food allergies are a subject of considerable public concern. Although food allergy is not the most common cause of adverse reactions to food, it is one of the best understood. Many symptoms can be related to food allergy, and reactions may be severe (Lessof, 1994).

Types of allergic reactions. Life-threatening or lethal anaphylactic reactions to food containing potent allergens (e.g. nuts) are well documented.

Any allergic reaction follows two separate phases: induction and elicitation. The characteristics of the antigens involved in these two phases may differ. The first phase corresponds to primary sensitization. It requires immunogenic antigens able to induce specific antibodies and results in the induction of specific memory cells. The second phase corresponds to elicitation, in which allergenic antigens are recognized by specific antibodies and provoke clinical allergic reactions.

Although several immunological mechanisms may operate in allergic reactions to food, the IgE-mediated immediate hypersensitivity reaction leads to the most severe adverse effects and is by far the best understood. The following classification established by Gell and Coombs distinguishes the different types of allergic reactions:

The Type I or anaphylactic reactions are mediated by IgE antibodies. Histamine is released through binding of the antigen to the antibody located on cell membranes of mast cells and basophils membrane.

The Type II or cytolytic reactions are mediated by IgG and IgM antibodies which fix complement, opsonize particles, or function in an antibody-dependent cellular cytotoxicity, leading to tissue damage.

The Type III or Arthus reactions are mainly mediated by IgG through a mechanism involving the generation of antigen–antibody complexes that subsequently fix complement, then deposit in the vascular endothelium where an inflammatory response occurs.

The Type IV or delayed hypersensitivity response is cell mediated. When sensitized T-lymphocytes come in contact with the sensitizing antigen, an inflammatory reaction is generated and lymphokines are produced, followed by an influx of granulocytes and macrophages. The classic example is skin contact sensitivity.

Low prevalence of food allergies. Type I allergies are rare and usually involve food proteins (Lessof, 1994). Allergy to food proteins occurs in less than 1–2% of the population (Bousquet *et al*., 1999; Metcalfe *et al*., 1996), while the prevalence of all adverse reactions to food additives is even lower, and estimated to be 0.03%–0.2% in Western

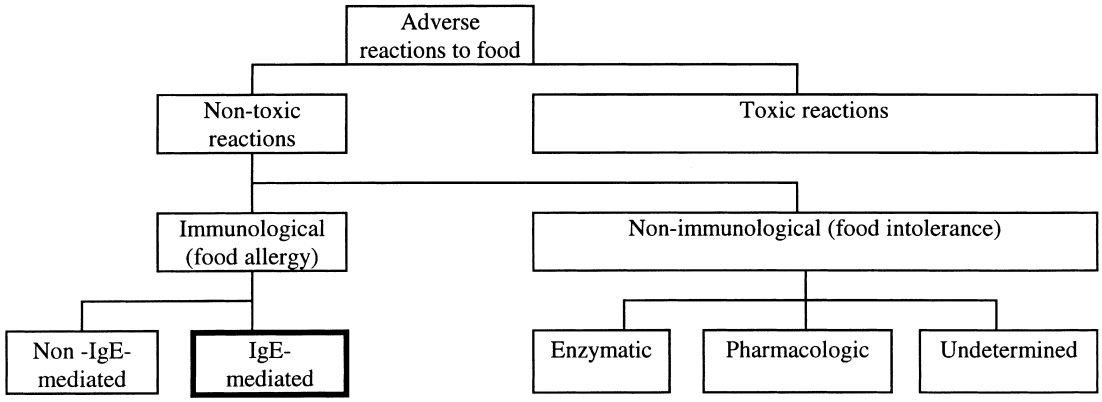


Fig. 4. Classification of adverse reactions to food according to the recommendations of the European Congress of Allergology and Clinical Immunology (from Wüthrich, 1995).

countries, 1–2% in children aged 5–16 years (Hannuksela and Haahtela, 1987; Kägi *et al.*, 1994; Madsen, 1995). Among the few adverse reactions to food additives, only a minority involves IgE-mediated mechanisms. It has been suggested that IgE may be implicated in sulfite-sensitive asthmatics. According to Lessof (1994), some alerts to food allergies have been shown to be related to idiosyncratic pharmacological reactions.

Pseudo-allergic reactions and irritancy. Pseudo-allergic reactions include adverse reactivity to polypeptide antibiotics and aminoglycosides, as well as compounds like intravenous anaesthetics and radiographic contrast media. There is no evidence of involvement of anaphylactic antibodies in these reactions (Stanworth, 1987). The mechanisms of pseudo-allergic reactions are varied, despite the similarities in the clinical manifestations. There is still much to be learnt about these reactions but evidence is growing that pseudo-allergies to at least some pharmaceutical agents result from an effect on the alternative complement pathway (Stanworth, 1987).

Irritancy may resemble allergy but is a pseudo-allergic reaction that does not involve immunological mechanisms. Chemical irritants such as formaldehyde, isocyanates, para-phenylenediamine (Dean and Murray, 1990), and food additives such as emulsifiers, acetic, ascorbic and lactic acids, potassium bicarbonate, potassium bromate and iodide, calcium acetate and sulfite (Hannuksela and Haahtela, 1987) act through sensory irritancy or toxic injury, causing direct stimulation of sensory receptors (Dean and Murray, 1990).

Identification of food allergies

The use of animal models to predict allergenicity and to establish a no-effect level for hypersensitivity may be limited in certain aspects. For example, the ability to produce IgE has been reported to vary among different strains of animals (SJL mice), underlying that allergic responses are highly dependent on genetic control. In studies where this genetic difference is observed, the dose of antigen and mode of administration are critical parameters (Roitt *et al.*, 1993). However, several European and US research groups are working on the development of animals models that still need to be validated, but which hold some promise; the Brown Norway rat and the C3H/HeSN mouse merit particular mention.

Risk assessment for allergenicity is also complicated by the mechanism of oral tolerance. Animals, including humans, develop oral tolerance to most ingested foreign proteins. Ingestion of proteins that are all antigenic in the recipient leads to an active immune regulation suppressing immune responses. Hence, this phenomenon constitutes a major difficulty in the development of animal models (i.e. difficulty to get an animal to develop an IgE response only when exposed to known allergens by the oral route).

The "gold standard" for unequivocally diagnosing a food allergy is a double-blind, placebo-controlled food challenge (DBPCFC) (Bousquet, 1994; Bousquet *et al.*, 1999). The best use of the DBPCFC is to confirm or refute a cause–effect relationship between allergic reaction and the specific food product. Any challenge tests should be conducted using the oral route of exposure, which corresponds to the relevant human exposure situation. However, challenge studies are seriously limited for the evaluation of allergy to chemicals in food because it is especially difficult to identify the specific ingredient responsible for an adverse reaction. Therefore, where formulations (i.e. flavours) are tested, allergy to a whole formulation is diagnosed rather than to an individual chemical (Taylor and Dormedy, 1998). As a consequence, it is rare to determine objectively whether a specific compound is associated with an IgE allergic reaction.

As explained before, allergic reactions follow two phases (i.e. induction and elicitation), each requiring particular characteristics of the antigens involved, in order to induce specific antibodies and provoke clinical allergic reactions. Consequently, the nature of the allergic reaction depends on specific factors

such as: the nature of the allergen, the route of exposure, and the exposure dose (Dewdney *et al*., 1991).

Nature of allergens

Main food allergens. Foods most commonly involved in allergies include cow's milk, egg, fish, crustaceans, peanuts and tree nuts, soybeans, wheat and sesame seeds (Bousquet *et al*., 1999; Dutch Health Council, 1991; FAO, 1995; Lessof, 1994).

Structural features that determine allergenicity. The structural features that determine allergenicity seem to follow the general rules that apply to immune responses. Among others, the size and foreigness of the molecule are determinant factors. The most potent allergens are foreign to the host and are large molecules such as proteins or other macromolecules. To elicit a specific IgE response, allergenic molecules must have a molecular weight ranging from 5 to 100 kDaltons. Thus, chemicals such as drugs and food additives are foreign compounds, but their molecular weight is usually too small to induce an allergic response. As IgE-mediated allergies are caused almost exclusively by proteins, for possible allergenic risks, the focus should be on the presence of protein in the substance under examination. Consequently, all real food allergens are big protein molecules and despite the variety of food proteins in the diet, only a few are likely to induce allergic sensitization, on rare occasions and in susceptible individuals (Metcalfe *et al*., 1996; Taylor *et al*., 1987).

The actual bioavailability of allergens is another determinant of allergic reactions. Indeed, one possible prerequisite to allergenicity is that protein allergens have to cross the mucosal membrane of the intestinal tract to elicit their effects. Therefore, in many cases a limiting factor for elicitation of allergic reactions is an intact tridimensional structure which allows protein allergens to reach and cross the intestinal mucosa. In addition to this limitation, the human digestive system displays an effective ability to remove these proteins before they reach the intestinal mucosa, through rapid proteolytic degradation, which greatly minimizes the opportunity for sensitization (Taylor *et al*., 1987).

For small molecules to become immunogenic, they must bind to carrier proteins and act as haptens (pro-allergens) (Metcalfe, 1996; Taylor *et al*., 1987). Thus, the degree of substitution of carrier proteins constitutes one more determinant for elicitation of allergy (Dean and Murray, 1990; Dewdney *et al*., 1991).

Ultimately, it should be noted that the binding of the chemical to carrier proteins may occur either in tissues or body fluids and is highly dependent on the proper protein reactivity of the chemical; that is, its ability to form covalent bounds with the carrier (Dewdney *et al*., 1991). Such structural characteristics are not common to all molecules.

Route of exposure

With respect to the allergic potential of a given chemical, all routes of exposure to the substance are not equivalent. To cite an example, penicillin administered by the oral route has been shown to be much less sensitizing than the parenteral route (Dewdney *et al*., 1991). Usually, inhalation is more implicated in allergies than the oral route since, as discussed above, chemicals such as food additives usually exhibit low molecular weights and are unlikely to induce IgE allergic reactions. Certain substances can act as haptens (such as plastics and resins, anhydrides, isocyanates, metals, cosmetics, textile finishes, etc.). But these are mostly involved in asthma (Type I) and/or contact dermatitis (Type IV) through inhalation or dermal contact (Dean and Murray, 1990). Therefore, the oral route of exposure is believed to be of little concern for such chemicals.

Discussion

Apart from the limiting factors described before (i.e. nature of the allergen, route of exposure, and certain genetic considerations), an allergic response is dependent on the exposure dose (Bousquet *et al*., 1999; Dewdney *et al*., 1991). In order to determine whether or not a threshold can be established for allergy, it would be useful to examine the exposure levels at which allergic reactions occur.

As already mentioned, an allergic reaction comprises a first phase corresponding to the primary sensitization, and a second one corresponding to the elicitation of clinical symptoms of allergy. For allergenic substances, there is a threshold dose for each individual at which sensitization occurs. This dose is thought to be relatively high, but for all allergens the actual value is unknown (Dutch Health Council, 1991). However, one should bear in mind that the various criteria described before (i.e. amount of allergen, size of the molecule, binding properties) need to be fulfilled in order to provoke a sensitization. These are factors limiting the probability that small molecules intended to be used in small amounts in the diet would bind to proteins, in a sufficient rate as to induce sensitization. In addition, as far as the route of exposure is concerned, sensitization through oral exposure is dependent on, and is limited by gastro-intestinal tract absorption as well as digestion.

Once sensitization has occurred, clinical symptoms will appear at a lower exposure level than that for sensitization. For most groups of allergenic foods listed by Bousquet *et al*. (1999), the amount of the product to induce the elicitation phase has not been identified. For eggs and egg products, an amount of 10 mg or lower per person has been described, whereas for fish and fish products and peanuts, soy bean and other legumes, respectively 6 mg or lower, and 0.1–1 mg per person is described (Bousquet *et al*., 1999). More clinical research is

needed to firmly establish threshold doses for specific allergenic foods. Thus, the actual threshold level of exposure is virtually unknown for all allergens, and a clear value cannot be predicted since the threshold dose of allergenic protein varies with time, and this should be emphasized again, with each individual, as reported for a group of well characterized subjects with peanut allergy (Hourihane *et al.*, 1997).

As inter-individual variability within the general and the sensitized populations is important, the traditional threshold approach has never been applied to food allergy, nor has a NOEL based on allergy ever been established. Therefore, the allergic risk for such substances is usually managed in other ways (e.g. labelling).

It should be mentioned that a Working Group convened by WHO is currently addressing this issue and will report back on the group's conclusions at the next JECFA meeting.

Conclusions

The present analysis was conducted to ascertain whether specific non-cancer toxicological endpoints, namely neurotoxicity, developmental neurotoxicity, immunotoxicity and developmental toxicity were more sensitive than those for structural class III of the Munro *et al.* (1996) database and whether the TTC of 1.5 μg/person/day derived from the cancer databases adequately covers such endpoints.

This analysis considerably expands previous analyses in regard to the concept of a TTC. In this respect, additional databases were developed for the toxicological endpoints neurotoxicity, developmental neurotoxicity, immunotoxicity, and developmental toxicity, and involved a detailed analysis of the data in relation to these endpoints.

The analysis indicated that, with the exception of neurotoxicity, there was no difference in the cumulative distribution of NOELs for the specific endpoints examined (i.e. developmental neurotoxicity and developmental toxicity) in comparison with the various toxicological endpoints for structural class III.

Although the cumulative distribution of NOELs for neurotoxic compounds was significantly lower than those for other non-cancer endpoints and structural class III, these substances were accommodated within the TTC of 1.5 μg/person/day. Furthermore, when comparing the distribution of 10^{-6} risks for carcinogens from the Gold *et al.* (1989) database with the distributions of NOELs for the endpoints examined in the present evaluation, it appeared that none of these specific non-cancer endpoints was more sensitive than cancer endpoints.

Analysis also demonstrated that, within the limitation of the database, the distribution of immune NOELs for the group of immunotoxicants examined here did not differ from the distribution of non-specific endpoints NOELs for the same compounds, showing that immunotoxicity should not be considered as an endpoint that is more sensitive than other non-cancer endpoints.

On the basis of the estimated dietary intakes of environmental oestrogenic chemicals (i.e. oestrogenic pesticides), it appears that the exposure to these chemicals is very low in comparison with exposure to naturally occurring phytoestrogens, and accounts for a negligible part of the total oestrogen burden from the diet. Such intake doses of environmental oestrogens are orders of magnitude lower than the exposure doses of known potent oestrogens reported to induce remarkable oestrogenic effects. Furthermore, as far as the oestrogenic potencies are concerned, scientific data show that environmental oestrogenic chemicals, in comparison with endogenous hormones, possess only little hormonal activity like phytoestrogens. Hence, the results do not suggest that hormonal effects are to be expected from the exposure to environmental oestrogenic chemicals found in foods. According to the present state of knowledge, the very low concentrations of such agents in food would not present a health risk, and the exposure threshold value of 1.5 μg/person/day would be sufficiently low to provide an adequate margin of safety against any adverse endocrine effect associated with dietary environmental substances.

The possibility of determining threshold doses for food allergens is currently being explored. As various criteria need to be satisfied before sensitization occurs, a threshold dose for sensitization is thought to be relatively high, although for all allergens this actual value is unknown, as is the threshold dose for elicitation of clinical symptoms. Threshold doses for sensitization and for elicitation vary with time and with each individual. Because of this important inter-individual variability, a threshold approach is not applied to food allergy and allergic risks are usually controlled by other means (i.e. labelling). However, if a threshold was to be envisaged, the amount of allergen, the size of the molecule and its binding properties, as well as the gastro-intestinal tract absorption would be factors limiting the probability that small molecules intended to be used in small amounts in the diet would bind to proteins, in a sufficient rate as to induce sensitization.

Consequently, on the basis of this analysis which was conducted adopting a "worst case" perspective and using conservative assumptions at each step of the procedure, it can be concluded that a TTC of 1.5 μg/person/day based on carcinogenesis is conservative, and provides adequate safety assurance. Chemical substances present in the diet that are consumed at levels below this threshold pose no appreciable risk. Moreover, for compounds which do not possess structural alerts for genotoxicity and carcinogenicity, further analysis may indicate that a higher TTC may be appropriate.

Annex 1

Neurotoxicity Database

Chronic and Subchronic Neurotoxicity Database

Substance	CAS no.	Species	no. days	Study type	Route	Doses
Pesticides/fungicides						
Amitraz	33089-61-1	rat	730	chr	fod	2.5; 10 mg/kg bw/day
azinphos methyl	86-50-0	rat	730	chr	fod	0; 2.5; 5; 20; 50 ppm in diet [c]
bidrin	141-66-2	rat	NG	3-gene. repro	fod	2; 5; 15; 50 ppm [b]
carbaryl	63-25-2	rat	50	sub	fod	10–20 mg/kg bw/day
carbofuran	1563-66-2	rat	28	sub	fod	0; 50; 200; 500; 1000; 3000; 6000 ppm [b]
chlorpyrifos	2921-88-2	rat	90	sub	gav	0; 0,1; 1; 5; 15 mg/kg bw/day
cismethrin	35764-59-1	rat	30	sub	gav	0; 6; 12; 18; 24 mg/kg bw/day
coumaphos	56-72-4	rat	730	chr	fod	0; 5; 10; 25; 100 ppm in diet [c]
crufomate	299-86-5	rat	600	chr	fod	0; 20; 40; 60; 80 ppm in diet [c]
deltamethrin	52918-63-5	rat	30	sub	gav	0; 2; 4; 6; 8 mg/kg bw/day
2,4-dichlorophenoxyacetic acid	94-75-7	rat	365	chr	fod	0; 5; 75; 150 mg/kg bw/day
dichlorvos	62-73-7	rat	730	chr	fod	0; 0.047; 0.46; 4.67; 46.7; 234 ppm in diet [a]
dimethoate	60-51-5	rat	730	chr	fod	0; 1; 5; 25; 100 ppm [a]
disulfoton	298-04-4	rat	730	chr	fod	0.05; 0.1 mg/kg bw/day
ethephon	16672-87-0	rat	728	chr	fod	15; 150 mg/kg bw/day
ethion	563-12-2	rat	NG	repro	NG	0.2; 1.025 mg/kg bw/day
ethyl-*p*-nitrophenylphenyl phosphorothioate	2104-64-5	hen	90	sub	gav	0.01; 0.1; 0.5; 1; 2.5; 5 mg/kg bw/day
fenchlorphos	299-84-3	rat	105	sub	fod	0; 0.5; 1.5; 5; 15; 50 mg/kg bw/day
fenpropathrin	39515-41-8	rat	NG	chr	fod	0; 125; 500 ppm [b]
fonophos	944-22-9	rat	730	chr	fod	0.5; 1.58 mg/kg bw/day
lindane	58-89-9	rat	90	sub	fod	2,5; 5 mg/kg bw/day
malathion	121-75-5	rat	730	chr	fod	5; 50 mg/kg bw/day
mancozeb	8018-01-7	rat	90	sub	fod	0; 20; 125; 750; 5000 ppm [b]
merphos	150-50-5	rat	112	sub	fod	0.1; 0.25 mg/kg bw/day
merphos oxide	78-48-8	rat	730	chr	fod	0.25; 1.25 mg/kg bw/day
methamidophos	10265-92-6	hen	90	sub	gav	0; 0,3; 1; 3 mg/kg bw/day
methidathion	950-37-8	rat	730	chr	fod	0.2; 2 mg/kg bw/day

parathion	56-38-2	rat	790	chr	fod	0; 50 ppm [b]
phosmet	732-11-6	rat	730	chr	fod	0; 20; 40; 400 ppm [b]
pirimiphos-methyl	29232-93-7	rat	730	chr	fod	0.5; 2.5 mg/kg bw/day
pydrin	51630-58-1	rat	91	sub	fod	0; 50; 150; 300; 500 ppm [b]
quinalphos	13593-03-8	mus	540	chr	fod	0.03; 0.15 mg/kg bw/day
tetrachlorvinphos	961-11-5	rat	730	chr	fod	6; 100 mg/kg bw/day
tetraethyldithiopyrophosphate	3689-24-5	rat	90	sub	fod	0; 5; 10; 20; 50 ppm [b]
thiram	137-26-8	rat	730	chr	fod	0; 100; 300; 1000; 2500 ppm [b]
trichlorphon	52-68-6	rat	90	sub	fod	16,25; 32,5 mg/kg bw/day
ziram	137-30-4	rat	91	sub	fod	0; 72; 210; 540 ppm [b]
Neuroactive agents						
cocaine hydrochloride	50-36-2	rat	16	subacu	gastric intub.	40; 80 mg/kg bw/day
Metals						
aluminium chloride	7429-90-5	rat	90	sub	orl	30; 100 mg/kg bw/day
methyl mercury	22967-92-6	rat	790	chr	gav	2; 10; 25; 50; 250μg/kg bw/day
bis(tri-*n*-butyltin)oxide (TBTO)	56-35-9	mus	10	terat	gav	0; 5; 10; 20; 30 mg/kg bw/day
Food additives						
butyl benzyl phthalate	85-68-7	rat	42	subacu	fod	500; 1500; 3000 mg/kg bw/day
Solvents						
n-butanol	71-36-3	rat	91	sub	gav	0; 30; 125; 500 mg/kg bw/day
Others						
acrylamide	79-06-1	rat	90	sub	orl	0; 0.05; 0.2; 1; 5; 20 mg/kg bw/day
3,3′-iminodipropionitrile (IDPN)	111-94-4	rat	105	sub	orl	0; 0.025; 0.05; 0.1; 0.2; 0.4% [c]

[continued]

Substance	Neurotoxic NOEL (mg/kg bw/day)	Neurotoxic LOEL (mg/kg bw/day)	Neurotoxic effect(s) reported	Reference
Pesticides/fungicides				
amitraz	2.5	10	neurobehavioural (nervous, aggressive & excitable behaviour)	Upjohn Co., 1973
azinphos methyl	0.18	0.36	inhibition of plasma ChE activity	Huntingdon Research Centre, 1966
bidrin	0.75	2.5	neurobehavioural (weakness, CNS effects)	Shell Chemical Co., 1965
carbaryl	none	10	neurobehavioural (altered motor activity, working memory & learning, decreased performance)	WHO, 1994
carbofuran	0.83*	10	neurobehavioural (decreased locomotor activity, splayed hindlimbs, tremors)	Freeman, 1993
chlorpyrifos	1.66*	15	neurobehavioural (alteration FOB, decreased motor activity)	Mattsson *et al*., 1996
cismethrin	2*	12	neurobehavioural (increased auditory startle)	Crofton and Reiter, 1984
coumaphos	0.4	0.8	inhibition of RBC and plasma ChE activity	Doull *et al*., 1960
crufomate	3	4	inhibition of RBC ChE activity	McCollister *et al*., 1968
deltamethrin	none	2	neurobehavioural (decreased motor activity & auditory startle amplitude)	Crofton and Reiter, 1984
2,4-dichlorophenoxyacetic acid	75	150	neurobehavioural (increased forelimb grip strength, FOB)	Mattsson *et al*., 1994
dichlorvos	0.23	2.3	inhibition of ChE activity	Shell Chemical Co., 1967
dimethoate	0.05	0.25	inhibition of brain, RBC and plasma ChE activity	American Cyanamid, 1986
disulfoton	0.05	0.1	inhibition of brain, RBC and plasma ChE activity	Mobay Chemical, 1975
ethephon	15	150	inhibition of RBC and plasma ChE activity	Union Carbide, 1978
ethion	0.2	1.025	inhibition of plasma ChE activity in females	FMC Corp., 1985
ethyl-*p*-nitrophenylphenyl phosphorothioate	0.003*	0.1	delayed neurotoxicity	Moribani, Nissan, du Pont Velsicol, 1982
fenchlorphos	5*	50	inhibition of ChE	McCollister *et al*., 1959
fenpropathrin	5	20	neurobehavioural (decreased performance inclined plane test)	Hend and Gellatly, 1980
fonophos	0.5	1.58	inhibition of RBC and plasma ChE activity	Stauffer Chemical Co., 1968
lindane	0.83*	5	neurobehavioural (increased EEG index, altered maze activity)	Desi, 1983
malathion	5	50	inhibition of brain ChE activity	American Cyanamid, 1980
mancozeb	2.73*	49.2	neurobehavioural (abnormal mobility, weakness), neurohistological	Stadler, 1991
merphos	0.03*	0.25	inhibition of RBC ChE activity in females	Virginia Carolina Chemical Corp., 1958

merphos oxide	0.25	1.25	inhibition of brain ChE activity	Mobay Chemical, 1969
methamidophos	0.1*	1	delayed neurotoxicity	Sachsse *et al.*, 1987
methidathion	0.2	2	inhibition of brain and RBC ChE activity	Ciba-Geigy, 1986
parathion	none	2.5	neurohistological (peripheral neuropathy, increased demyelination)	Daly, 1984
phosmet	2	20	inhibition of RBC and plasma ChE activity	Stauffer Chemical Co., 1967
pirimiphos-methyl	0.5	2.5	inhibition of plasma ChE activity	ICI Americas, Inc., 1973
pydrin	0.83*	7.5	neurobehavioural (abnormal locomotor activity, uncoordination, hypersensitivity to sound, convulsions)	Shell Chemical Co., 1984
quinalphos	0.03	0.15	inhibition of plasma ChE activity	Sandoz Inc., 1980
tetrachlorvinphos	6	100	inhibition of RBC ChE activity	Shell Chemical Co., 1966
tetraethyldithiopyrophosphate	0.16*	1	inhibition of RBC and plasma ChE activity	Kimmerle *et al.*, 1974
thiram	5	15	neurobehavioural (weakness, ataxia, hindlimb paralysis), neuromorphological	duPont, 1954
trichlorphon	5.41*	32.5	neurobehavioural (increased EEG index, altered maze activity)	Desi, 1983
ziram	4.66*	34	brain neurotoxic esterase (nofx neurobehavioural observed on FOB, motor activity)	Nemec, 1993
Neuroactive agents				
cocaine hydrochloride	none	40	neurobehavioural (increased locomotor activity & stereotypic behaviours)	Dow-Edwards *et al.*, 1989
Metals				
aluminium chloride	none	30	neurobehavioural (decreased learning ability & choline-acetyltransferase activity, increased acetylcholinesterase activity)	Bilkei-Gorzo, 1993
methyl mercury	0.05	0.25	neurohistological	Munro *et al.*, 1980
bis(tri-*n*-butyltin)oxide (TBTO)	5	10	neurobehavioural (altered maternal behaviour)	Baroncelli *et al.*, 1995
Food additives				
butyl benzyl phthalate	500*	3000	neuromuscular dysfunction (hindlimb stiffness)	Robinson, 1991
Solvents				
n-butanol	41.66*	500	neurobehavioural (hypoactivity, ataxia)	US EPA, 1986
Others				
acrylamide	0.066*	1	neurohistological (nerve damage)	Burek *et al.*, 1980
3,3′-iminodipropionitrile (IDPN)	10*	60	neurobehavioural (altered motor activity), neurohistological (axonopathies)	Llorens and Rodriguezfarre, 1997

*A NOEL from a subchronic study was divided by 3 to adjust for differences between subchronic and chronic studies (see text for explanation).

Acute Neurotoxicity Database

Substance	CAS no.	Species	no. days	Study type	Route	Doses
Pesticides/fungicides						
aldicarb	116-06-3	rat	1	acu	ip	0; 0.266 mg/kg bw/day
amitrole	61-82-5	rat	1	acu	gav	100–3000 mg/kg bw/day
bifenthrin	82657-04-3	rat	5	acu	gav	0; 1; 3; 10; 30 mg/kg bw/day
bitertanol	55179-31-2	rat	1	acu	gav	3–1000 mg/kg bw/day
carbendazim	10605-21-7	hen	1	acu	gav	500; 2500; 5000 mg/kg bw/day
chlorfenvinphos	470-90-6	rat	1	acu	gav	1; 2 mg/kg bw/day
cypermethrin	52315-07-8	rat	7	acu	gav	0; 25; 50; 100; 150; 200 mg/kg bw/day
α-cypermethrin		rat	1	acu	gav	0; 4; 20; 40 mg/kg bw/day
dieldrin	60-57-1	rat	1	acu	gav	0; 0.5; 1.5; 4.5 mg/kg bw/day
diniconazole	83657-18-5	rat	1	acu	gav	75–600 mg/kg bw/day
etridazole	2593-15-9	rat	1	acu	gav	187–1500 mg/kg bw/day
flumethrin		rat	1	acu	gav	0; 10; 31.5; 100 mg/kg bw/day
flutriafole	76674-21-0	rat	1	acu	gav	500–2000 mg/kg bw/day
hexaconazole	79983-71-4	rat	1	acu	gav	500–2000 mg/kg bw/day
isazophos	67329-04-9	rat	1	acu	gav	6–50 mg/kg bw/day
mevinphos	7786-34-7	rat	1	acu	gav	0; 0.025; 0.1; 2; 3.5 mg/kg bw/day
paclobutrazole	76738-62-0	rat	1	acu	gav	75–1000 mg/kg bw/day
parathion methyl	298-00-0	rat	1	acu	gav	0; 0.025; 7.5; 10 mg/kg bw/day
penconazole	66246-88-6	rat	1	acu	gav	20–2000 mg/kg bw/day
permethrin	52645-53-1	rat	1	acu	gav	50–200 mg/kg bw/day
p,p′-DDT	50-29-3	rat	1	acu	gav	15–75 mg/kg bw/day
propiconazole	60207-90-1	rat	1	acu	gav	125–1000 mg/kg bw/day
tebuconazole	107534-96-3	rat	1	acu	gav	50–2000 mg/kg bw/day
tebufenozide		rat	1	acu	gav	0; 500; 1000; 2000 mg/kg bw/day
tebuthiuron	34014-18-1	rat	1	acu	gav	125–500 mg/kg bw/day
triadimefon	43121-43-3	rat	1	acu	gav	50–400 mg/kg bw/day
triadimenol	55219-65-3	rat	1	acu	gav	50–400 mg/kg bw/day
tricyclazole	41814-78-2	rat	1	acu	gav	1–30 mg/kg bw/day
Neuroactive agents						
imipramine	50-49-7	rat	1	acu	gav	10–100 mg/kg bw/day
maprotiline	10262-69-8	rat	1	acu	gav	10–100 mg/kg bw/day
methamphetamine hydrochloride	300-42-5	rat	1	acu	sc	3; 4; 5 mg/kg bw/4 x daily
milnaciplan hydrochloride (TN-912)		rat	1	acu	gav	10-100 mg/kg bw/day
Metals						
tributyltin chloride	56573-85-4	rat	1	acu	gav	0; 6.3; 12.5; 25; 50 mg/kg bw/day
triethyltin bromide		rat	4	acu	gav	0; 1; 2; 3 mg/kg bw/twice weekly
trimethyltin chloride	1066-45-1	rat	1	acu	gastric intub.	0; 5; 6; 7 mg/kg bw/day
trimethyltin hydroxide	56-24-6	rat	1	acu	gav	0; 9 mg/kg bw/day
Others						
3-nitropropionic acid	504-88-1	rat	1	acu	ip	10; 15; 20 mg/kg bw/day

[continued]

Substance	Neurotoxic NOEL (mg/kg bw/day)	Neurotoxic LOEL (mg/kg bw/day)	Neurotoxic effect(s) reported	Reference
Pesticides/fungicides				
aldicarb	none	0.266	neurobehavioural (decreased ability standard avoidance test)	Johnson and Carpenter, 1966
amitrole	3000	none	nofx neurobehavioural (motor activity)	Crofton, 1996
bifenthrin	10	30	neurobehavioural (alertness, locomotor activity, apathy, tremor, abnormal gait)	Algate *et al.*, 1985
bitertanol	1000	none	nofx neurobehavioural (motor activity)	Crofton, 1996
carbendazim	2500	5000	delayed neurotoxicity	Goldenthal, 1978
chlorfenvinphos	1	2	neurological (prominent arousal pattern in spontaneous electroencephalographic activity)	Osumi *et al.*, 1975
cypermethrin	50	100	neurobehavioural (ataxia, splayed hindlimb, hyperexcitability to auditory stimuli), neurochemical	Rose and Dewar, 1983
α-cypermethrin	4	20	neurobehavioural (alterations FOB, increased motor activity)	Fokkema, 1994
dieldrin	0.5	1.5	neurobehavioural (deficit escape response)	Carlson and Rosellini, 1987
diniconazole	600	none	nofx neurobehavioural (motor activity)	Crofton, 1996
etridazole	1500	none	nofx neurobehavioural (motor activity)	Crofton, 1996
flumethrin	10	31.5	neurobehavioural (increased motor activity)	Starke, 1985
flutriafole	2000	none	nofx neurobehavioural (motor activity)	Crofton, 1996
hexaconazole	2000	none	nofx neurobehavioural (motor activity)	Crofton, 1996
isazophos	50	none	nofx neurobehavioural (motor activity)	Crofton, 1996
mevinphos	0.1	2	neurobehavioural (impaired mobility & gait, bizarre behaviour, altered reflexes, clonic & tonic convulsions, tremors)	Lamb, 1993
paclobutrazole	1000	none	nofx neurobehavioural (motor activity)	Crofton, 1996
parathion methyl	0.025	7.5	neurobehavioural (altered motor activity & function)	Minnema, 1994
penconazole	2000	none	nofx neurobehavioural (motor activity)	Crofton, 1996
permethrin	50	60	neurobehavioural (increased amplitude auditory startle response)	Crofton and Reiter, 1988
p,*p*′-DDT	25	50	neurobehavioural (increased amplitude & sensitization to auditory startle)	Crofton and Reiter, 1988
propiconazole	1000	none	nofx neurobehavioural (motor activity)	Crofton, 1996
tebuconazole	2000	none	nofx neurobehavioural (motor activity)	Crofton, 1996
tebufenozide	2000	none	nofx neurobehavioural (FOB, motor activity)	Swenson *et al.*, 1994
tebuthiuron	500	none	nofx neurobehavioural (motor activity)	Crofton, 1996
triadimefon	50	100	neurobehavioural (hyperactivity figure-8 maze)	Crofton, 1996
triadimenol	50	100	neurobehavioural (hyperactivity figure-8 maze)	Crofton, 1996

tricyclazole	30	none	nofx neurobehavioural (motor activity)	Crofton, 1996
Neuroactive agents				
imipramine	none	10	neurofunctional (altered EEG pattern)	Kawasaki *et al*., 1991
maprotiline	none	10	neurofunctional (altered EEG pattern)	Kawasaki *et al*., 1991
methamphetamine hydrochloride	none	12	neurochemical (decreased dopamine, dihydroxyphenylacetic acid, homovanillic acid, serotonin, 5-hydroxyindolacetic acid)	Abekawa *et al*., 1997
milnaciplan hydrochloride (TN-912)	none	10	neurofunctional (altered EEG pattern)	Kawasaki *et al*., 1991
Metals				
tributyltin chloride	6.3	12.5	neurobehavioural (decreased spontaneous motor activity, inhibited shock avoidance response)	Ema *et al*., 1991
triethyltin bromide	none	0.28	neurobehavioural (decreased startle response), neurohistological	Squibb *et al*., 1980
trimethyltin chloride	6	7	neurobehavioural (increased activity in figure-8 maze)	Ruppert *et al*., 1982
trimethyltin hydroxide	none	9	neurobehavioural (learning and aggression impairment), neurochemical	Ishida *et al*., 1997
Others				
3-nitropropionic acid	10	15	neurobehavioural (decreased amplitude, latency and pre-pulse inhibition in auditory startle response)	Kodsi and Swerdlow, 1997

Annex 2

Developmental Neurotoxicity Database

Substance	CAS no.	Species	Period of exposure	no. days	Study type	Route	Doses
Antiproliferative agents							
5-azacytidine	320-67-2	mus	GD 7	1	terat	ip	100–1000 μg/kg bw/day
colchicine	64-86-8	mus	GD 6-8	3	terat	ip	0.5–1 mg/kg bw/day
cytocine arabinoside		mus	GD 8-11, GD 14-15	4; 2	terat	ip	30 mg/kg bw/day
hydroxyurea	127-07-1	rat	GD 9-12	4	terat	ip	415–1125 mg/kg bw/day
methylazoxymethanol	590-96-5	rat	GD 15	1	terat	ip	20 mg/kg bw/day
Neuroactive agents (drugs & others)							
chlorpromazine	50-53-3	rat	GD 6-15	10	terat	gav	0; 1; 3; 9 mg/kg bw/day
cocaine hydrochloride	50-36-2	rat	last 2 weeks of G	14	terat	gastric intub.	30; 60 mg/kg bw/day
δ-9-tetrahydrocannabinol	8063-14-7	rat	GD 2-22	21	terat	gastric intub.	15; 50 mg/kg bw/day
diazepam	439-14-5	rat	GD 13-20	8	terat	gav	1; 5 mg/kg bw/day
fenfluramine hydrochloride		rat	GD 7-20	14	terat	gastric intub.	0; 20 mg/kg bw/day
fluoxetine		rat	GD 7-20	14	terat	orl	0; 1; 5; 12 mg/kg bw/day
imipramine	50-49-7	rat	GD 8-20	13	terat	sc	0; 5; 10 mg/kg bw/day
methadone hydrochloride	1095-90-5	rat	GD 8-22	15	terat	gastric intub.	0; 5; 10 mg/kg bw/day
pergolide mesylate	66104-23-2	mus	GD 15-PND 20	NG	terat	gav	0; 0.002; 0.1; 3 mg/kg bw /day
phenobarbital	50-06-6	rat	GD 7-18	12	terat	gav	5; 50; 80 mg/kg bw/day
phenytoin	630-93-3	mus	PND 2-4	3	terat	gav	10; 17.5; 25; 35 mg/kg bw/day
prochlorperazine edisylate	1257-78-9	rat	GD 7-20	14	terat	gastric intub.	0; 25 mg/kg bw/day
propoxyphene hydrochloride	1639-60-7	rat	GD 7-20	14	terat	gastric intub.	0; 75 mg/kg bw/day
trimethadione	127-48-0	rat	GD 7-18	12	terat	gav	5; 50; 250 mg/kg/day
Pesticides							
amitraz	33089-61-1	rat	GD 1, 4, 7, 10, 13, 16, 19	7	terat	gav	20 mg/kg bw/day
bioallethrin	584-79-2	mus	PND 10-16	7	develop	gav	0; 0.21; 0.42; 0.7; 42 mg/kg bw/day
carbaryl	63-25-2	rat	3-months old	1	develop	gav	10; 50 mg/kg bw/day
deltamethrin	52918-63-5	mus	PND 10-16	7	develop	gav	0; 0.7 mg/kg bw/day
diazinon	333-41-5	mus	G	NG	terat	gav	0; 0.18; 9 mg/kg bw/day
2,4-dichlorophenoxyacetic acid (2,4-D)	94-75-7	rat	PND 7-17; PND 12-25	11; 14	develop	gav	70; 100 mg/kg bw/day
emamectin benzoate		rat	GD 6-LD 20	NG	terat	gav	0; 0.1; 0.6; 2.5 mg/kg bw/day
ivermectin	71827-03-7	rat	GD 6-20, LD 2-20	15; 19	terat	gav	1; 2; 4 mg/kg bw/day
sumithion	3344-14-7	rat	GD 7-15	9	terat	gav	5; 10; 15 mg/kg bw/day

Metals							
aluminium	7429-90-5	rat	G	NG	terat	gav	0; 400 mg/kg bw/day
cadmium chloride	10108-64-2	rat	25 days before M, M, G	NG	repro	gav	0.04; 0.4; 4 mg/kg bw/day
lead acetate	15347-57-6	rat	weanling	NG	chr	orl	0; 25 ppm [c]
methyl mercury	22967-92-6	mok	G, L, to 7 years old	NG	develop	fod	25; 50 μg/kg bw/day
bis(tri-*n*-butyltin)oxide (TBTO)	56-35-9	rat	GD 6-20	15	terat	gastric intub.	0; 2.5; 5; 10 mg/kg bw/day
Environmental pollutants							
Arochlor 1254 (PCB)	11097-69-1	rat	G, L	NG	terat	fod	0.02; 2.5; 26; 269 ppm [b]
2,2′,4,4′,5,5′ hexabromobiphenyl (PBB)	59536-65-1	rat	GD 6- PND 24	NG	terat	gav	0; 0.2; 2 mg/kg bw/day
3,3′,4,4′-tetrachlorobiphenyl (PCB 77)		rat	GD 7-18	12	terat	gav	0; 1 mg/kg bw/day
smokeless tobacco (nicotine)	54-11-5	rat	GD 6-20	15	terat	gav	0; 1.33; 4; 6 mg/kg bw/day
Food additives							
albendazole	54965-21-8	rat	60 days before mating, M	NG	repro	gav	0; 1; 10; 30 mg/kg bw/day
amaranth	915-67-3	mus	Pre-Mating, M, G, L	NG	repro	orl	0; 0.025; 0.075; 0.225% [a]
brominated vegetable oil		rat	2 wks before M, M, G, L	NG	repro	fod	0; 0.25; 0.5; 1; 2 % diet [c]
butylated hydroxytoluene (BHT)	128-37-0	rat	M, G, L	NG	repro	fod	0.125; 0.25; 0.5% diet [b]
glutamate monosodium	142-47-2	mus	GD 17-21	5	terat	gav	2.5; 4 mg/g bw/day [c]
sodium nitrite	7632-00-0	rat	Pre-Mating, M, G, L - PND 90	NG	terat & repro	fod	0; 0.0125; 0.025; 0.05% diet [a]
sodium salicylate	54-21-7	rat	GD 8-10	3	terat	gav	0; 125; 175 mg/kg bw/day
Solvents							
2-methoxyethanol	109-86-4	rat	GD 7-18	12	terat	gav	0.006; 0.012; 0.025% liquid diet [a]
toluene	108-88-3	mus	G, L	NG	terat	orl	16; 80; 400 ppm [c]
trichloroethylene	79-01-6	rat	G, PND 1-21	NG	terat	orl	312; 625; 1250 mg/l [c]
Natural toxins							
fumonisin (FB1)		rat	PND 3-72	70	develop	sc	0; 0.4; 0.8 mg/kg bw/day
ochratoxin A	303-47-9	rat	GD 11-14	4	terat	gav	0.5 mg/kg bw/day
secalonic acid D mycotoxin	35287-69-5	mus	GD 13	1	terat	gav	0; 15; 25 mg/kg bw/day
Others							
acrylamide	79-06-1	rat	GD 6-LD 10	NG	terat	gav	0; 5; 10; 15; 20 mg/kg bw/day
retinoic acid	302-79-4	rat	GD 14-16	3	terat	fod	2; 2.5; 4; 5; 6 mg/kg bw/day

Substance	Dev. neurotoxic NOEL (mg/kg bw/day)	Dev. neurotoxic LOEL (mg/kg bw/day)	Developmental neurotoxic effect(s) reported	Reference
Antiproliferative agents				
5-azacytidine	none	0.1	neuromorphological (exencephaly)	Matsuda, 1990
colchicine	none	0.5	neurodevelopmental (neuromorphological & neurohistological)	Sieber *et al.*, 1978
cytocine arabinoside	none	30	neurobehavioural (figure-8 maze activity, general behavioural development)	Gray *et al.*, 1986
hydroxyurea	none	415	neurodevelopmental (neuromorphological & neurohistological)	Chaube and Murphy, 1966
methylazoxymethanol	none	20	neurodevelopmental (neuromorphological & neurohistological)	Kabat *et al.*, 1985
Neuroactive agents (drugs & others)				
chlorpromazine	1	3	neurobehavioural (increased activity in open field, decreased reflex latency time)	Robertson *et al.*, 1980
cocaine hydrochloride	30	60	neurobehavioural (increased ontogeny of motor activity)	Hutchings *et al.*, 1989a
δ-9-tetrahydrocannabinol	50	none	nofx neurobehavioural (tested on: activity, rest-activity cycle)	Hutchings *et al.*, 1989b
diazepam	1	5	neurobehavioural (altered habituation behaviour)	Shore *et al.*, 1983
fenfluramine hydrochloride	none	20	neurobehavioural (delayed locomotor development, increased activity in open field, decreased latency to begin exploration), neuromorphological (decreased brain wt)	Vorhees *et al.*, 1979
fluoxetine	12	none	nofx neurobehavioural (tested on: locomotor activity, acoustic startle, startle, spontaneous alternation, passive avoidance, complex learning in water maze)	Vorhees *et al.*, 1994
imipramine	none	5	neurobehavioural (decreased auditory startle habituation amplitude, increased locomotor activity, faster negative geotaxic test), neurochemical	Ali *et al.*, 1986
methadone hydrochloride	10	none	nofx neurobehavioural (tested on: lever-pressing response, auditory-visual discrimination, learning, inhibitory response)	Hutchings *et al.*, 1979
pergolide mesylate	3	none	nofx neurobehavioural (tested on: negative geotaxis, activity, auditory startle, active avoidance)	Buelke-Sam *et al.*, 1991
phenobarbital	50	80	neurobehavioural (delayed swimming ontogeny)	Vorhees, 1983
phenytoin	17.5	25	neuromorphological (reduced brain weight, increased number pyknotic cells, EGL thicker), was more concervative than neurobehavioural	Ohmori *et al.*, 1997
prochlorperazine edisylate	none	25	neurobehavioural (decreased rotorod performance)	Vorhees *et al.*, 1979

propoxyphene hydrochloride	none	75	neurobehavioural (increased activity in open field and active avoidance response, decreased rotorod performance), neurohistological (increased hippocampus cell counts)	Vorhees *et al*., 1979
trimethadione	50	250	neurobehavioural (delayed swimming ontogeny, figure-8 maze activity and spontaneous alternation behaviour)	Vorhees, 1985
Pesticides				
amitraz	20	none	nofx neurobehavioural (tested on: surface righting and startle development, locomotor activity and rearing frequency in open field)	Palermo-Neto *et al*., 1994
bioallethrin	none	0.21	neurobehavioural (increased locomotor and rearing activity)	Ahlbom *et al*., 1994
carbaryl	10	50	neurobehavioural (decreased frequency ambulation in open field)	Takahashi *et al*., 1991
deltamethrin	none	0.7	neurobehavioural (increased locomotor activity)	Eriksson and Fredriksson, 1991
diazinon	none	0.18	neurobehavioural (delayed contact placing & sexual maturity, reduced angle/inclined plane test	Spyker and Avery, 1977
2,4-dichlorophenoxyacetic acid (2,4-D)	none	70	neurochemical , neuromorphological (decreased brain wt)	Rosso *et al*., 1997
emamectin benzoate	0.6	2.5	neurobehavioural (open field motor activity, auditory startle, passive avoidance)	Wise *et al*., 1997
ivermectin	none	1	neurobehavioural (altered cliff avoidance, locomotion, negative geotaxis & swimming development)	Poul, 1988
sumithion	5	10	neurobehavioural (extinction conditioned escape response)	Lehotszy *et al*., 1989
Metals				
aluminium	none	400	neurobehavioural (decreased performance in negative geotaxis test, altered locomotor co-ordination & operant conditioning tests)	Muller *et al*., 1990
cadmium chloride	0,013	0.4	neurobehavioural (decreased exploratory locomotor activity)	Baranski *et al*., 1983
lead acetate	none	31	neurobehavioural (increased overall response rate)	Cory-Slechta *et al*., 1985
methyl mercury	none	0.025	neurobehavioural (disrupted alternation performance , sensory-motor deficits, loss of vibration sensitivity)	Rice, 1992
bis(tri-*n*-butyltin)oxide (TBTO)	5	10	neurobehavioural (altered motor activity development)	Crofton *et al*., 1989
Environmental pollutants				
Arochlor 1254 (PCB)	0.001	0.13	neurobehavioural (delayed auditory startle development & ontogeny of negative geotaxis, air-righting)	Overmann *et al*., 1987
2,2′,4,4′,5,5′ hexabromobiphenyl (PBB)	0.2	2	neurobehavioural (locomotion development, cliff avoidance, cage emergence, open field activity)	Henck *et al*., 1994
3,3′,4,4′-tetrachlorobiphenyl (PCB 77)	none	1	neurobehavioural (impaired passive avoidance behaviour, increased latency in catalepsy test)	Weinandharer *et al*., 1997

[continued]

smokeless tobacco (nicotine)	4	12	neurobehavioural (delayed surface righting & swimming development, increased activity in open field)	Cole *et al.*, date unknown
Food additives				
albendazole	30	none	nofx neurobehavioural	Boutemy, 1980
amaranth	none	54.4	neurobehavioural (altered cliff avoidance, surface righting, olfactory orientation)	Tanaka, 1993
brominated vegetable oil	166.5	333	neurobehavioural (severe impairment in FOB)	Vorhees *et al.*, 1983
butylated hydroxytoluene (BHT)	125	250	neurobehavioural (increased surface righting time, decreased activity in open field, altered passive avoidance, delayed fore-limb swimming development)	Brunner *et al.*, 1978
glutamate monosodium	none	2500	neurobehavioural (impaired Y-maze discrimination learning)	Yu *et al.*, 1997
sodium nitrite	10.75	21.5	neurobehavioural (decreased open-field activity, delayed swimming development)	Vorhees *et al.*, 1984
sodium salicylate	none	125	neurobehavioural (increased locomotor activity)	Buelke-Sam *et al.*, 1984
Solvents				
2-methoxyethanol	16	32	neurobehavioural (increased errors number in water maze)	Nelson *et al.*, 1989
toluene	2.04	10.2	neurobehavioural (decreased open field activity, surface righting response)	Kostas and Hotchin, 1981
trichloroethylene	80	160	neurobehavioural (increased locomotor activity in running wheel)	Taylor *et al.*, 1985
Natural toxins				
fumonisin (FB1)	0.4	0.8	neurochemical, neurohistological (decreased myelination)	Kwon *et al.*, 1997
ochratoxin A	none	0.5	neurobehavioural (learning deficits)	Kihara *et al.*, 1984
secalonic acid D mycotoxin	15	25	neurobehavioural (delayed ontogeny swimming, decreased norepinephrine and dopamine levels)	St Omer and Bolon, 1990
Others				
acrylamide	10	15	neurobehavioural (decreased horizontal motor activity and auditory startle response)	Wise *et al.*, 1995
retinoic acid	2	2.5	neurobehavioural (decreased M-maze performance)	Nolen, 1986

Annex 3

Immunotoxicity Database

Substance	CAS no.	Species	no. days	Study type	Route	Doses	Immune NOEL (mg/kg bw/day)	Non-immune NOEL	Immune LOEL	Non-immune LOEL
Oral administration										
benzidine	92-87-5	mus	5	subacu	gav	cf. Luster *et al.* (1992, 1993)	11	none	NG	2.7
2,4-diaminotoluene	95-80-7	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	none	none	25	24
dimethylvinyl chloride	513-37-1	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	none	none	50	125
DPH	630-93-3	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	150	50	NG	NG
ethylene dibromide	106-93-4	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	none	30	125	NG
hexachlorodibenzo-*p*-dioxin	19408-74-3	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	0.06	1.00E-05	NG	NG
lithium carbonate	554-13-2	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	none	none	50	98
nitrobenzene	98-95-3	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	30	none	NG	60
m-nitrotoluene	99-08-1	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	none	none	200	40
p-nitrotoluene	99-99-0	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	400	200	NG	NG
pentachorophenol	87-86-5	mus	14	subacu	fod	cf. Luster *et al.* (1992, 1993)	10	3	NG	NG
o-phenylphenol	90-43-7	mus	10 over 14	subacu	gav	cf. Luster *et al.* (1992, 1993)	100	10	NG	NG
TCDD (2,3,7,8-tetrachloro-dibenzo-*p*-dioxin)	1746-01-3	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	none	none	0.086	1.00E-05
tetraethyl lead	78-00-2	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	0.5	none	NG	0.0012
4,4-thiobis (6-*tert*-butyl-*m*-cresol)	96-69-5	mus	14	subacu	gav	cf. Luster *et al.* (1992, 1993)	none	none	10	45
Non-oral administration										
azathioprine	446-86-6	mus	22 over 30	subacu	ip	cf. Luster *et al.* (1992, 1993)	none	none	10	1
benzo[*a*]pyrene	50-32-8	mus	14	subacu	sc	cf. Luster *et al.* (1992, 1993)	none	none	50	50
diethylstilboestrol	56-53-1	mus	14	subacu	sc	cf. Luster *et al.* (1992, 1993)	none	none	0.2	1.00E-05
DMB(a)A	57-97-6	mus	14	subacu	sc	cf. Luster *et al.* (1992, 1993)	none	none	5	1.25
ethyl carbamate	51-79-6	mus	14	subacu	ip	cf. Luster *et al.* (1992, 1993)	2	none	NG	15
indomethacin	53-86-1	mus	5	subacu	sc	cf. Luster *et al.* (1992, 1993)	2	1.6	NG	NG
N-nitrosodimethylamine	62-75-9	mus	14	subacu	ip	cf. Luster *et al.* (1992, 1993)	none	none	1.5	5
ochratoxin A	303-47-9	mus	8	subacu	ip	cf. Luster *et al.* (1992, 1993)	none	none	3.4	0.0625

[continued]

phorbol myristate acetate (TPA)	16561-29-8	mus	4 over 14	subacu	sc	cf. Luster *et al.* (1992, 1993)	20	none	NG	0.32
Additional data/oral administration										
arochlor 1248 (PCB)	12672-29-6	mus	35	subacu	fod	50; 100; 500; 1000 ppm [c]	90	3.22	180	8
arochlor 1254 (PCB)	11097-69-1	rat	105	sub	gastric intub.	0.1; 1; 10; 25 mg/kg bw/day	1	0.002	10	0.25
benzene	71-43-2	mus	28	subacu	orl	166 mg/l [c]	none	none	41	2400
cocaine	50-36-2	rat	5	subacu	gav	15–60 mg/kg bw/day	none	30	15	60
δ-9-tetrahydrocannabinol §	1972-08-3	rat	26	subacu	gav	6; 12 mg/kg bw/day	none	50	6	none
2′,3′-dideoxyadenosine §	4097-22-7	mus	22	subacu	gav	0; 87, 5; 175; 350 mg/kg bw/day	none	175	87.5	350
2′,3′-dideoxyinosine		mus	180	sub	gav	100; 250; 500; 1000 mg/kg bw/day	none	500	100	1000
nickel sulfate	7786-81-4	mus	180	sub	orl	0; 2; 5 g/l [c]	none	264	528	1321
tri-*n*-butyltin oxide	56-35-9	rat	540	chr	fod	0; 0,5; 5; 50 ppm [b]	0.025	0.19	0.25	2.1
trichloroethylene	79-01-6	mus	180	sub	gav	0.1; 1; 2,5; 5 mg/ml [a]	none	2688	24	NG
triphenyltin hydroxide	76-87-9	rat	28	subacu	fod	0; 25 mg/kg bw/day	none	none	25	13
Additional data/non-oral administration										
arsine §	7784-42-1	rat	65	sub	inh	0; 0.025; 0,5; 2,5; 5 ppm [c]	0.015	0.3	0.3	1.5
ginseng §		rat	10	subacu	sc	0; 25 mg/kg bw/day	none	none	25	50

§Substance originally from the Luster's database, for which a lower and more recent NOEL was retrieved and recorded.

Substance	Immunotoxic effect(s) reported	Reference	Non-immunotoxic endpoint	Reference non-immune NOEL & LOEL
Oral administration				
benzidine	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	neural, hepatic	Littlefield *et al.*, 1983
2,4-diaminotoluene	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro	Thysen *et al.*, 1985
dimethylvinyl chloride	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	splenic	NTP, 1986
DPH	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	teratogenic	McClain and Langhoff, 1979
ethylene dibromide	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	mortality	Teramoto *et al.*, 1980
hexachlorodibenzo-*p*-dioxin	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro	Murray *et al.*, 1979
lithium carbonate	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro, bw, hepatic, renal	Ibrahim and Canolty, 1990
nitrobenzene	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro	Kawashima *et al.*, 1995
m-nitrotoluene	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	hepatic	NTP, 1992
p-nitrotoluene	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	hepatic, splenic	Burns *et al.*, 1994
pentachorophenol	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	hepatic, renal	Schwetz *et al.*, 1978
o-phenylphenol	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	blood	Luster *et al.*, 1981
TCDD	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro	Murray *et al.*, 1979
tetraethyl lead	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	hepatic, thymus	Schepers, 1964
4,4-thiobis (6-*tert*-butyl-*m*-cresol)	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	hepatic	NTP, 1994
Non-oral administration				
azathioprine	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro (ip)	Scott, 1977
benzo[*a*]pyrene	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro (sc)	Bui *et al.*, 1986
diethylstilboestrol	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro (sc)	McLachlan, 1977
DMB(a)A	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro (orl)	Davis *et al.*, 1978
ethyl carbamate	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro (sc)	NTIS, 1968
indomethacin	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro/vascular permeability (sc)	Hoos and Hoffman, 1983
N-nitrosodimethylamine	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro (ip)	Chaube, 1973
ochratoxin A	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	renal (gav)	NTP, 1989
phorbol myristate acetate (TPA)	cf. Luster *et al.* (1992, 1993)	Luster *et al.* (1992, 1993)	repro (sc)	Nagasawa *et al.*, 1980
Additional data/oral administration				
Arochlor 1248 (PCB)	decreased resistance to *Salmonella* + endotoxin	Thomas and Hinsdill, 1978	bdw	Thomas and Hinsdill, 1980
Arochlor 1254 (PCB)	thymus wt, decreased NK cell activity	Smialowicz *et al.*, 1989	neurobehavioral and somatic effects	Overmann *et al.*, 1987

[continued]

benzene	mult (leuko- & lymphopaenia, thy. atrophy, suppressed B & T cell mutagenesis, antibody production/T-dependent antigen (sRBC)	Hsieh *et al.*, 1990	neoplastic effects	Authors unknown, *Toxicol Appl Pharmacol* 1986; **82**, 19
cocaine	inhibition PFC and DTH	Watson *et al.*, 1983	neurobehavioral	Hutchings *et al.*, 1989a
δ-9-tetrahydrocannabinol §	decreased spleen wt, immunosuppression	Luthra *et al.*, 1980	neurobehavioral	Hutchings *et al.*, 1989b
2′,3′-dideoxyadenosine §	Ig(+), PFC, Listeria	Cao *et al.*, 1990	bdw	Luster *et al.*, 1989
2′,3′-dideoxyinosine	suppressed humoral immune response, suppressed response to T-dependent antigen (sRBC)	Phillips *et al.*, 1997	bdw	Phillips *et al.*, 1997
nickel sulfate	thymus wt, LPS	Luster *et al.,* 1992, 1993	bdw, owt, metabolic disruption	Dieter *et al.*, 1988
tri-*n*-butyltin oxide	immunosuppression (decreased thymus wt and IgE titers, increased T. spiralis larvae)	Vos *et al.*, 1990	mult (bdw, bld, owt)	Wester *et al.*, 1988
trichloroethylene	inhibited cell-mediated immunity (dose-dependent) & bone marrow stem cell colonization	Sanders *et al.*, 1982	repro	Authors unknown, *Toxicologist* 1984; **4**,179
triphenyltin hydroxide	decreased spleen wt, suppressed delayed hypersensitivity responses & splenic lymphocytes resp. to T-cell mitogen	Vos *et al.*, 1984	bdw (maternal), ltl (fetal)	Chernoff *et al.*, 1990
Additional data/non-oral administration				
arsine§	decreased Thy 1.2, CD4, CD8	Luster *et al.,* 1992, 1993	bdw	Morrissey *et al.*, 1990
ginseng§	decreased serum IgM & IgA levels, reduced bacterial load and mast cells number in lungs	Song *et al.*, 1997	hypoglycemic (decreased blood glucose	Wang *et al.*, 1990

§Substance originally from the Luster's database, for which a lower and more recent NOEL was retrieved and recorded.

Annex 4

Developmental Toxicity Database

Substance	CAS no.	Species	Study type	Route	Doses
acephate	30560-19-1	rat	2-gene. repro	fod	0; 50; 500 ppm [b]
acesulfame potassium	33665-90-6	rat	multigene. repro	fod	0; 0.3; 1; 3 % [c]
acrylic acid	79-10-7	rat	2-gene. repro	orl	0; 500; 2500; 5000 ppm [b]
alar	1596-84-5	rat	3-gene. repro	fod	0; 300 ppm [b]
albendazole	54965-21-8	rat	2-gene. repro	gav	0; 1; 10; 30 mg/kg bw/day
aldicarb	116-06-3	rat	3-gene. repro	gastric intub.	0; 0.2; 0,3; 0,7 mg/kg bw/day
alkylate 215		rat	2-gene. repro	fod	0; 5; 50; 500 mg/kg bw/day
amaranth	915-67-3	rat	3-gene. repro	fod	30; 300; 3000; 30000 ppm [a]
amitraz	33089-61-1	rat	3-gene. repro	fod	15; 50 ppm [b]
Arochlor 1016 (PCB)	12674-11-2	mok	repro	fod	0; 0.25; 1 ppm [b]
Arochlor 1254 (PCB)	11097-69-1	mus	terat	fod	0; 1;10;100 ppm [b]
atrazine	1912-24-9	rat	2-gene repro	gav	0; 10; 50; 500 ppm [b]
avermectin B1	65195-55-3	rat	2-gene. repro	fod	0; 0.05; 0,12; 0,4 mg/kg bw/day
baythroid	68359-37-5	rat	3-gene. repro	fod	50; 150 ppm [b]
benomyl	17804-35-2	rat	3-gene. repro	fod	0; 100; 500; 2500 ppm [b]
bidrin	141-66-2	rat	3-gene. repro	fod	2; 5; 15; 50 ppm [b]
brominated vegetable oil		rat	repro	orl	0; 0.25; 0,5; 1; 2 % diet [c]
2-butanol	78-92-2	rat	multigene. develop.	fod	0; 0,3; 1; 2; 3 % [b]
butylate	2008-41-5	rat	2-gene. repro	fod	0; 200; 1000; 4000 ppm [b]
butylated hydroxytoluene (BHT)	128-37-0	rat	repro	fod	0; 25; 100; 500 mg/kg bw/day
captan	133-06-2	rat	3-gene. repro	fod	0; 25; 100; 250; 500 mg/kg bw/day
carbosulfan	55285-14-8	rat	3-gene. repro	orl	1; 12.5 mg/kg bw/day
2-chlorophenol	95-57-8	rat	sub	fod	0; 5; 50; 500 ppm [b]
chlorpyrifos	2921-88-2	rat	2-gene. repro	fod	0; 0,1; 1; 5 mg/kg bw/day
cyclohexylamine hydrochloride	108-91-8	rat	2-year multigene.	gav	15; 50; 100; 150 mg/kg bw/day
cyclophosphamide	50-18-0	rat	repro	fod	3.4; 5,1 mg/kg bw/day
cyhalothrin/karate	68085-85-8	rat	3-gene. repro	orl	0; 10; 30; 100 ppm [b]
2,4-dichlorophenol	120-83-2	rat	repro	fod	3; 30; 300 ppm [b]
di(2-ethylhexyl)adipate	103-23-1	rat	repro	orl	0; 28; 170; 1080 mg/kg bw/day
dimethyldicarbonate (DMDC)	4525-33-1	rat	repro	fod	0; 4000 ppm [c]
dinoseb	88-85-7	rat	3-gene. repro	fod	0; 1; 3; 10 mg/kg bw/day

[continued]

disodium 5′-inosinate	4691-65-0	rat	3-gene. repro	fod	0; 0.5; 1; 2% [c]
EDTA disodium	139-33-3	rat	2-year repro	fod	0; 50; 125; 250 mg/kg/day
endothall	145-73-3	rat	3-gene. repro	fod	100; 2500 ppm [b]
ethion	563-12-2	rat	3-gene. repro	fod	4; 25 ppm [b]
erythritol	149-32-6	rat	2-gene. repro	fod	0; 2.5; 5; 10% [a]
ethylenediamine	107-15-3	rat	2-gene. repro	fod	0; 50; 150; 500 mg/kg bw/day
ethyl maltol	4940-11-8	rat	repro	fod	50; 100; 200 mg/kg bw/day
express	101200-48-0	rat	2-gene. repro	fod	25; 250 ppm [b]
fenpropathrin	39515-41-8	rat	multigene. repro	fod	0; 40; 120; 360 ppm [b]
flurprimidol	56425-91-3	rat	2-gene. repro	fod	0; 25; 100; 1000 ppm [b]
flutolanil	66332-96-5	rat	3-gene. repro & terat	fod	0; 1000; 10000 ppm [b]
folpet	133-07-03	rat	2-gene. repro	fod	690; 3200 ppm [b]
fosetyl-al	39148-24-8	rat	3-gene. repro	fod	6000; 12000 ppm [b]
glyphosate	1071-83-6	rat	3-gene. repro	fod	0; 3; 10; 30 mg/kg bw/day
haloxyfop-methyl	69806-40-2	rat	3-gene. repro	fod	0; 0.005; 0.05; 1 mg/kg bw/day
heptachlor	76-44-8	rat	3-gene. repro	fod	10 ppm [b]
heptachlor epoxide	1024-57-3	dog	2-gene. repro	fod	1; 3 ppm [b]
hexachlorobenzene	118-74-1	rat	2-gene. repro	fod	0; 0.32; 1.6; 8; 40 ppm [b]
hexachlorophene	70-30-4	rat	3-gene. repro	fod	20; 60 ppm [b]
hydroquinone	123-31-9	rat	2-gene. repro	gav	0; 15; 50; 150 mg/kg bw/day
iprodione	36734-19-7	rat	3-gene. repro	fod	500; 2000 ppm [b]
ipronidazole	14885-29-1	rat	3-gene. repro	fod	0; 20; 200; 2000 ppm [b]
lactofen	77501-63-4	rat	2-gene. repro	fod	50; 500 ppm [b]
linuron	330-55-2	rat	3-gene. repro	fod	25; 125; 625 ppm [b]
losartan		rat	2-gene. repro	gav	25; 100; 200 mg/kg bw/day
methoxychlor	72-43-5	rat	3-gene. repro	fod	0; 200; 1000 ppm [b]
methyl bromide	74-83-9	rat	2-gene. repro & develop.	fod	80; 200; 500 ppm (total bromine) [a]
methyl carbamate (MC)	598-55-0	rat	repro	orl	0; 0.5; 2.5; 12.5; 62.5 mg/kg bw/day
methyl ethyl ketone (MEK)	78-93-3	rat	multigene. develop.	orl	0; 0.3; 1; 2; 3 % [b]
metolachlor	51218-45-2	rat	2-gene. repro	fod	0; 30; 300; 1000 ppm [b]
napropamide	15299-99-7	rat	3-gene. repro	fod	0; 10; 30; 100 mg/kg bw/day
norflurazon	27314-13-2	rat	3-gene. repro	fod	375; 1025 ppm [b]
patulin	149-29-1	rat	repro	gastric intub.	0; 0.1; 0.5; 1.5 mg/kg bw–3 times/week
prochloraz	67747-09-5	rat	2-gene. repro	fod	150; 625 ppm [b]
propargite	2312-35-8	rat	3-gene. repro	fod	0; 100; 300 ppm [b]
ronidazole	7681-76-7	rat	2-gene. repro	fod	0; 0.02; 0.04; 0.089% [b]
rotenone	83-79-4	rat	2-gene. repro	fod	0; 7.5; 37.5; 75 ppm [b]
sethoxydim	74051-80-2	rat	2-gene. repro	fod	360; 1080; 3240 ppm [b]
sodium lauryl glyceryl ether sulfonate		rat	repro	fod	0.1; 0.5% [d]

sodium lauryl trioxyethylene sulfate	13150-00-0	rat	repro	fod	0.1; 0.5% [d]
sucrose polyester		rat	2-gene. repro	fod	1%. 5%. 10% [c]
sulfur mustard (bis(2-chloroethyl)sulfide)	505-60-2	rat	2-gene. repro	gav	0; 0.03; 0.1; 0.4 mg/kg bw/day
systhane	88671-89-0	rat	2-gene. repro	gav	2.32; 9.28; 46.4 mg/kg bw/day
tebuthiuron	34014-18-1	rat	2-gene. repro	fod	0; 100; 200; 400 ppm [b]
thiabendazole	148-79-8	rat	2-gene. repro	fod	10., 30, 90 mg/kg bw/day
T2-toxin		mus	2-gene. repro	fod	0; 1.5; 3 ppm [c]
1,2,4-trichlorobenzene	120-82-1	rat	multigene. repro	orl	0; 25; 100; 400 ppm [b]
1,1,1-trichloroethane	71-55-6	mus	multigene. repro	orl	0; 100; 300; 1000 mg/kg bw/day
tridiphane	58138-08-2	rat	2-gene. repro	fod	0; 1; 5; 30 mg/kg bw/day
zearalenone	17924-92-4	rat	2-gene. repro	fod	0; 0.1; 1; 10 mg/kg bw/day

Substance	Authors' develop. NOEL (mg/kg bw/day)	Calculated develop. NOEL (mg/kg bw/day)	Develop. LOEL (mg/kg bw/day)	Effect(s) reported in the pups	Reference
acephate	2.5	2.5	25	decreased pup viability	Chevron Chemical Co., 1987
acesulfame potassium	3% diet	2751	none	nofx	Sinkeldam *et al*., 1976
acrylic acid	none	240	460	histological changes in stomach	BASF, 1993
alar	15	15	none	nofx	Uniroyal Chemical, 1966a
albendazole	none	1	10	hypoplastic seminiferous ducts	Boutemy, 1980
aldicarb	none	0.7	none	nofx (bdw was not included)	Union Carbide, 1974
alkylate 215	5	5	50	early lethality	Robinson and Schroeder, 1992
amaranth	2420	2420	none	nofx	Collins *et al*., 1975
amitraz	1.6	1.6	5	early lethality (during suckling)	Boots Hercules Agrochemicals Co., 1980
Arochlor 1016 (PCB)	0.007	0.007	0.028	neurobehavioural	Schantz *et al*., 1989, 1991
Arochlor 1254 (PCB)	12.5	12.5	none	nofx	Welsch, 1985
atrazine	none	34.97	none	nofx (bdw was not included)	Ciba-Geigy, 1987
avermectin B1	0.12	0.12	0.4	mult (increased retinal folds, decreased pup viability)	Merck and Co., 1984
baythroid	2.5	2.5	7.5	decreased pup viability	Mobay Chemical, 1983
benomyl	none	125	none	nofx (bdw was not included)	du Pont, 1968
bidrin	0.1	0.1	0.25	decreased pup viability	Shell Chemical Co., 1965
brominated vegetable oil	166.5	333	none	neurobehavioural	Vorhees *et al*., 1983
2-butanol	1771	1771	3122	msk, ren	Cox *et al*., 1975
butylate	10	10	50	owt (kidney and brain weights)	Stauffer Chemical Co., 1986
butylated hydroxytoluene (BHT)	25	100	25	mult (thyroid hyperactivity, hepatic enzymes induction)	Olsen *et al*., 1986
captan	none	500	none	nofx (bdw and con were not included)	Chevron Chemical Co., 1982
carbosulfan	1	1	12.5	decreased pup viability	FMC Corp., 1982
2-chlorophenol	5	1.66	50	increased number stillborns	Exon and Koller, 1982
chlorpyrifos	none	1	5	early lethality	Breslin *et al*., 1996
cyclohexylamine hydrochloride	100	150	100	mucosal thickening bladder, renal calcification	Oser *et al*., 1976
cyclophosphamide	none	3.4	5.1	decreased fertility	Hales *et al*., 1992
cyhalothrin/karate	none	1.5	5	decreased pup viability (bdw was not included)	Coopers Chemical Industries, 1984
2,4-dichlorophenol	0.3	0.3	3	immune toxicity (delayed-type hypersensitivity responses)	Exon and Koller, 1985
di(2-ethylhexyl)adipate	170	170	none	decreased number pups per litter (bdw was not included)	ICI, 1988
dimethyldicarbonate (DMDC)	none	590	none	nofx	Eiben *et al*., 1983
dinoseb	none	10	none	nofx (bdw was not included)	Dow Chemical Co., 1981
disodium 5′-inosinate	none	2964	none	nofx	Palmer *et al*., 1975
EDTA disodium	none	250	none	nofx	Oser *et al*., 1963

endothall	5	5	125	ren (kidney and adrenal discoloration), early lethality	Pennwalt Corp., 1965
ethion	0.2	0.2	1.25	plasma ChE inhibition	FMC Corp., 1985
erythritol	none	7600	none	nofx (increased food intake was not included)	Waalkens-Berendsen *et al.*, 1996
ethylenediamine	none	150	500	owt (decreased liver weight and increased kidney weight)	Yang *et al.*, 1984
ethyl maltol	none	200	none	nofx	Gralla *et al.*, 1969
express	1.25	1.25	12.5	owt (decreased spleen weight)	du Pont, 1986
fenpropathrin	3	3	9	tremors, lethality	JMPR 1993, n° 233-255
flurprimidol	1.8	1.8	7.3	hepatocellular changes (fatty change and vacuolation)	Eli Lilly and Co., 1986
flutolanil	none	63.7	661.8	owt (increased liver weight) (bdw was not included)	Nor-Am Chemical Co., 1982
folpet	34.5	34.5	160	decreased male fertility	Chevron Chemical Co., 1985
fosetyl-al	300	300	600	urinary tract changes	Rhone-Poulenc, 1981
glyphosate	10	10	30	ren (increased renal tubular dilation)	Monsanto Co., 1981
haloxyfop-methyl	0.005	0.005	0.05	owt (decreased kidney weights), decreased fertility	Dow Chemical, 1985
heptachlor	0.5	0.5	none	nofx	Velsicol Chemical, 1967
heptachlor epoxide	0.025	0.025	0.075	liv (liver lesions in pups)	Velsicol Chemical, 1973
hexachlorobenzene0.08	0.08	0.29	liv		Arnold *et al.*, 1985
hexachlorophene	1	1	3	decreased number corpora lutea, early lethality (during suckling)	Kalo Laboratories Inc., 1979
hydroquinonenone	none	50	150	nofx (bdw was not included)	Blacker *et al.*, 1993
iprodione	25	25	100	owt (decreased kidney weights)	Rhone-Poulenc, 1976
ipronidazole	10	10	100	repro (damage of testes tubules, loss of spermatogenesis)	Dale, 1976
lactofen	2.5	2.5	25	mult (owt, liv, bld)	PPG Industries, 1983
linuron	none	6.25	31.25	liv (atrophy), owt (liver, kidney)	du Pont, 1984
losartan	none	25	100	early lethality (bdw was not included)	Spence *et al.*, 1995
methoxychlor	10	10	50	decreased fertility and pup viability	du Pont, 1966
methyl bromide	41.6	41.6	none	nofx (bdw and con were not included)	Kaneda *et al.*, 1993
methyl carbamate (MC)	none	62.5	none	nofx	Steinhoff *et al.*, 1977
methyl ethyl ketone (MEK)	1771	1771	3122	msk, ren	Cox *et al.*, 1975
metolachlor	15	15	50	owt (liver and thyroid to bdw ratios)	Ciba-Geigy, 1981
napropamide	none	100	none	nofx (bdw was not included)	Stauffer Chemical Co., 1978
norflurazon	18.75	18.75	51.25	decreased fertility and pup viability	Sandoz Inc., 1975
patulin	none	0.64	none	nofx (bdw was not included)	Becci *et al.*, 1981
prochloraz	7.5	7.5	31.25	increased number stillborns	Nor-Am Chemical Co., 1981

[continued]

propargite	15	15	none	nofx	Uniroyal Chemical, 1966b
ronidazole	none	60	none	nofx	Wazeter *et al.*, 1969
rotenone	none	1.88	3.8	decreased number pups per litter (bdw was not included)	US Fish and Wildlife Service, 1983
sethoxydim	18	18	54	owt (increased thyroid and adrenal weights)	BASF, 1980
sodium lauryl glyceryl ether sulfonate	none	199	none	nofx	Tusing *et al.*, 1962
sodium lauryl trioxyethylene sulfate	none	199	none	nofx	Tusing *et al.*, 1962
sucrose polyester	none	14736	none	nofx	Nolen *et al.*, 1987
sulfur mustard (bis(2-chloroethyl)sulfide)	0.1	0.1	0.4	acanthosis (thickening of squamous mucosa with hyperkeratosis)	Sasser *et al.*, 1996
systhane	2.32	2.32	9.28	liv (hepatocellular hypertrophy), owt (increased liver weight)	Rohm and Haas Co., 1985
tebuthiuron	none	28	none	nofx (bdw was not included)	Elanco Products, 1981
thiabendazole	none	90	none	nofx (bdw, con were not included)	Wise *et al.*, 1993
T2-toxin	none	0.76	none	nofx	Rousseaux *et al.*, 1986
1,2,4-trichlorobenzene	14.8	14.8	53.6	owt (increased adrenal weights), vacuolization of zona fasciculata in cortex	Robinson *et al.*, 1981
1,1,1-trichloroethane	none	1000	none	nofx	Lane *et al.*, 1982
tridiphane	0.33	0.33	1.67	decreased fertility	Dow Chemical, 1984
zearalenone	0.1	0.1	1	msk, owt (thyroid, pituitary and adrenal glands)	Becci *et al.*, 1982

Abbreviations used in the Databases

acu = acute study (up to 48 hr)
bld = blood effects
chr = chronic study (over 365 days)
con = food consumption
cvs = cardiovascular effects
fod = diet
G = gestation
GD = gestation day (s)
gastric intub. = gastric intubation
gav = gavage
ip = intraperitoneal
kid = renal effects
L = lactation
LD = lactation day (s)
liv = hepatic effects
ltl = lethal, or lethality
M = mating
mok = monkey
msk = musculo-skeletal
mult = multiple effects
mus = mouse
NA = not applicable
NG = not given
nofx = no effects
nos = non-specific effects
orl = drinking water
owt = organ weight changes
PND = postnatal day (s)
repro = reproductive effects, or reproduction study
sc = subcutaneous
sub = subchronic study (60 to 365 days)
subacu = subacute study (48 hr to 59 days)
terat = teratogenic effects, or teratology study
[a] = authors of the original study provided the dose converted in mg/kg bw/day
[b] = in the JECFA, JMPR or IRIS monographs, JECFA, JMPR or US EPA provided the dose converted in mg/kg bw/day
[c] = conversions were calculated using standard values
[d] = doses in mg/kg bw/day were calculated from data provided by the authors.

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