Health Canada Santé Canada

DETERMINATION OF "TOXIC"

FOR THE PURPOSES OF THE NEW SUBSTANCES PROVISIONS (CHEMICALS AND POLYMERS) UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT

HUMAN HEALTH CONSIDERATIONS

Canada

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This approach to human health risk assessment for new chemicals and polymers under section 64(c) of the *Canadian Environmental Protection Act* (CEPA) and the interpretation of the concept of "suspected to be toxic" was prepared by Jacqueline Sitwell, Shaunalea Savard and Ranjan Bose, all of the New Substances Assessment and Control Bureau, Health Canada.

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Introduction

The *Canadian Environmental Protection Act* (CEPA) was assented to on June 28, 1988 (CEPA, 1988), and the revised act was assented to on September 14, 1999 (CEPA,1999). The renewed CEPA provides the federal government authority regarding pollution prevention and protection of the environment and human health in order to contribute to sustainable development. The Act addresses pollution problems on land, in water, and through all layers of the atmosphere. The approach for dealing with new substances under CEPA is preventative in nature. The provisions for Substances New to Canada, in Part 5 of the Act (Part 2 under CEPA, 1988), are intended to ensure that no new substance is introduced into the Canadian marketplace before an assessment of whether it is "toxic" or "capable of becoming toxic" has been completed.

For the purposes of Part 5 of CEPA, 1999, the attribute "toxic" is defined in section 64 of the Act as follows:

"... A substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that

(a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;

(b) constitute or may constitute a danger to the environment on which life depends; or

(c) constitute or may constitute a danger in Canada to human life or health."

The definition of toxic encompasses both hazard (the intrinsic toxicity of a substance) and exposure to that substance; therefore, it can be regarded as being the same as the concept of risk. The definition is consistent with the Risk Management Policy that was implemented throughout the former Health Protection Branch of Health Canada, and it continues to be consistent with the decision-making framework currently used in Health Canada.

A substance that has been determined to be toxic may be added to the List of Toxic Substances in Schedule I of the Act, and may be regulated under section 91 of CEPA.

The following measures under section 84 of CEPA, 1999 may be taken when the government suspects that a substance is toxic or capable of becoming toxic:

(a) permit any person to manufacture or import the substance, subject to any conditions that the Ministers may specify;

(b) prohibit the manufacture or import of the substance for a period not exceeding two years (this prohibition lapses at the end of this two-year period unless, before the end of this period, a notice of proposed regulation under section 91 of CEPA is published in the *Canada Gazette*); or

(c) prohibit the manufacture or import of the substance until supplementary information or test results have been submitted to the government and assessed.

Measures under section 84 of CEPA must be taken by the government before the expiration of the assessment period. The assessment period for a new chemical or polymer specified in the New Substances

Notification Regulations Parts I and II (1994) ranged from 5 to 90 calendar days, depending on the category of the substance. The revised regulations that came into force in 2005 provide assessment periods ranging from 5 to 75 calendar days. This period can be extended once, for a duration that cannot exceed the original number of assessment days.

It is necessary both to carry out the risk assessment and to implement measures for the management of any anticipated risks within the assessment period for new substances; consequently, appropriate action must be identified before the assessment period ends. This document includes some general considerations that are used to support risk management decisions designed to protect members of the general (i.e. non-occupationally exposed) public. These assessments and management decisions are not intended to address the health risks arising from the possible use of these substances in settings that would be regulated by other federal or provincial statutes.

This document describes how the New Substances Program in Health Canada has interpreted and implemented the definition of toxic in section 11 of CEPA, 1988 during the first several years of the implementation of the New Substances Notification (NSN) provisions and Regulations, as well as during the current implementation of section 64 of CEPA, 1999. Toxicity defined under CEPA is essentially the same as "risk," a term used in this document and in the assessment of new substances by the NSN Program. The new substances provisions of CEPA also apply to biotechnology products, and there is a separate set of notification regulations for living organisms, including microorganisms, biochemicals and biopolymers. Criteria for determining when a living organism is toxic or is suspected to be toxic are not described in this document.

Definition of Toxic

The definition of toxic under CEPA (1988 as well as 1999) for assessing the risks posed to human life or health takes into account that:

- both harmful effects and level of exposure are considered;
- the harmful effects can be in relation to human health or the environment;
- the term "constitutes or may constitute a danger" indicates that the evidence of harm could be based on effects observed directly in humans or in relevant animal studies;
- the definition specifies a danger in Canada, therefore, a substance existing elsewhere that is not transported through the environment to Canada cannot be designated toxic; and
- the term "... is entering or may enter ..." indicates that the substance exists in the environment at present, or could exist in the environment if a given set of circumstances is met.

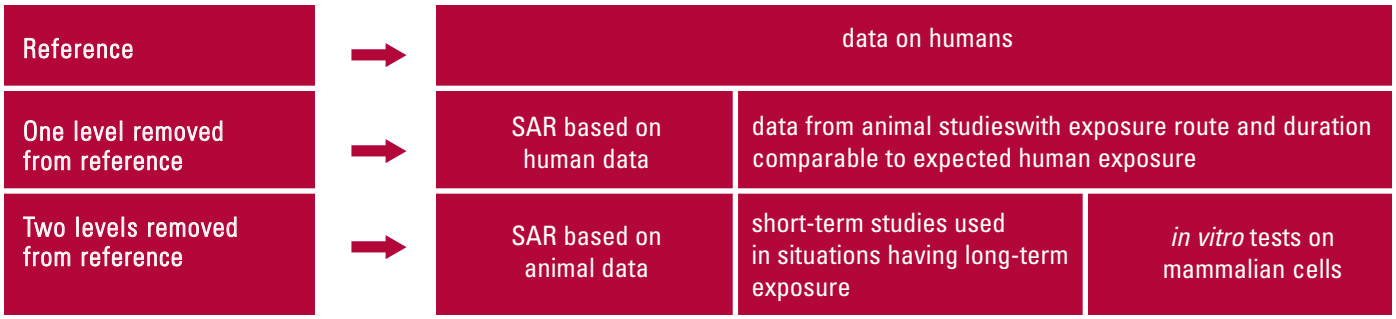
Guiding Principles

The New Substances Notification Regulations prescribe both technical and administrative information that must be provided to the government before a new substance can be manufactured in, or imported into, Canada. In addition to submitted data, information may be available from other sources, such as

the scientific literature, notifications made under section 70 of CEPA, 1999 (previously section 17 under CEPA, 1988), and from structurally related substances. Information that must be made available on a new substance will, in general, be more limited than that required for a comprehensive assessment of potential health effects and exposure. Therefore, the new substances provisions of CEPA allow control measures to be taken if a substance is suspected of being toxic as well as if it is toxic.

The amounts, types and quality of evidence generally required to determine that a substance is toxic, as defined in CEPA, are often different from those required for classifying a substance under the term "suspected of being toxic." A substance suspected of being toxic may be surrounded by a degree of uncertainty about the precise nature of the adverse health effects or about exposure because the evaluation is conducted during the pre-market or early market phases of commerce (in the case of new substances). Examples of uncertainty include the difficulty in determining the actual human health hazard implications of adverse health effects observed during toxicological tests conducted in animals, the use of data from short-term studies to predict chronic effects, the extrapolation of results at high exposures in tests to low exposures in the environment, or the use of environmental fate models to

Figure 1. Levels of uncertainty associated with toxicological information



predict levels of a substance in the environment. Another example of uncertainty is that introduced by the use of structural activity relationships (SARs) to predict toxicological or other properties of a substance. The levels of uncertainty associated with different types of toxicological data on a substance are illustrated in Figure 1; this schema is used as a guide in determining whether a substance is toxic as defined in CEPA or is suspected of being toxic.

Toxicological information from human studies of high quality or derived from sources one level removed from this reference level can be used to support a determination of toxic as defined in CEPA. The conclusion regarding toxicity will depend on whether or not the potential level of exposure is below that for which the health risk is considered significant, or for which the health risk is considered negligible. Toxicological information that is two levels removed from the reference level can be used to support a determination that a substance is suspected of being toxic. Again, the conclusion will also depend on the exposure assessment.

Two additional situations arise in which a substance may be suspected of being toxic even though only one of the two components that define risk is used or is available: (1) when there is insufficient information available to determine whether or not there is a health hazard, but where the potential for

exposure to a substance is unusually high and is of a serious concern; and (2) when the information on health hazards of a substance is of a serious nature, but it is not possible to estimate the factors needed to carry out an exposure assessment. Identification of the potential for high exposure levels may be based on, for example, continuous release of high quantities, persistence in the environment, or presence in widely used consumer products. Serious health hazards include reproductive effects, developmental effects and carcinogenicity.

Human Exposure Assessment

To determine whether a substance is toxic under CEPA, it is necessary to assess the exposure of the general population to that substance. Monitoring information for the levels of a new substance in air, drinking water, food, dust, soil or sediment in Canada will typically not be available; therefore, these levels will need to be estimated. Confidence in exposure assessment based on predicted environmental levels will normally be less than an assessment based on monitoring data; nevertheless, predicted data can be used to support the conclusion that a substance is toxic if a reasonable set of assumptions and methods is used.

Exposure of the general public to chemicals and polymers can occur by direct or indirect means. Direct exposure has been defined as resulting from direct contact with, or close proximity to, the chemical during any part of its lifecycle, whether knowingly or not (OECD, 1982). Furthermore, direct human exposure is distinguished from indirect human exposure in that no significant pathway in the environment intervenes between the point of release and the point of human exposure. However, it is not always possible to differentiate between the two cases.

In the first few years of the New Substances Program, exposure assessments were carried out for the average adult males and females in the Canadian population, and also for members of subpopulations if they were determined to be at greater risk (e.g. children, pregnant women, those who consume large quantities of certain types of food that could concentrate the substance). Exposures are now estimated routinely for all age groups. Intake is estimated from all possible sources, including food, air, water, dust/soil and products, through ingestion, inhalation and dermal absorption. Standard intake values for drinking water, food, air, dust and soil, and standard body weights and body surface areas for the Canadian population are used to estimate total intake of a substance (Health Canada, 1993; personal communication: memo to Ron Newhook from Mike Walker, Dec. 18, 1998).

DIRECT EXPOSURE

Direct human exposure to consumer and commercial chemicals occurs mainly by inhalation and/or dermal contact. Several factors are considered in the assessment of direct exposure to a substance. These include intended use; physical state of substance and medium (solid, liquid, gas); physical form (dust, fume, mist, etc); availability of the chemical if it is present in association with other substances; vapour pressure of the chemical; estimated stability; particle-size distribution; duration and frequency of exposure; and rate of release of the chemical (e.g. evaporation rate) (OECD, 1982).

Use scenarios are considered in estimating direct exposure to a new substance. The new substance can be compared with chemicals or products already on the market for which exposure information is available.

Use scenarios for the assessment of new substances consider intended uses, and in some circumstances, reasonably envisaged other uses. Misuses and accidental releases are not considered.

Standard scenarios have been compiled using information from surveys on the use of consumer products, experimental data and other published data (Versar, 1986). Computer-based exposure models that incorporate scenarios have been developed. One such model enables exposure through inhalation to be estimated for the user of a product and for a person in another room of the home (Consumer Exposure Model, Versar, 1991). The types of rooms, occupancy patterns and other parameters are selected judiciously. Examples of consumer exposure scenarios include general purpose cleaners, latex paints, fabric protectors, aerosol paints, laundry detergents, solid air-fresheners, bar soaps, and used motor oil.

ENVIRONMENTAL (INDIRECT) EXPOSURE

Substances can enter the general environment through industrial waste streams, from releases from intended industrial uses, air emissions, household wastewater and landfill sites. Substances used in the home environment could move from septic systems to ground water or to wastewater treatment facilities and then to surface water and eventually to ground water. Rivers, lakes and ground water are all sources of drinking water.

Environmental exposure occurs through contaminants present in food, drinking water, domestic and recreational water, air, dust and soil. An estimate of environmental exposure should take into account release, dispersion and transformation in the environment. Computer-based models can assist in estimating the physical–chemical properties and the distribution and/or concentrations of a substance in the environment (which depend on the properties). Several computer software models,[1] which are available commercially, can estimate environmental fate and distribution in various media.

A useful computer-based model, developed for the United States Environmental Protection Agency (U.S. EPA) by Versar (1989), can estimate the intake through drinking water and ingestion of fish of a chemical in a consumer product that has been disposed of in household wastewater.

Hazard Identification

GENERAL PRINCIPLES

The assessment of toxicological test data on a new substance is intended to permit an assessment of the potential hazard a substance may pose to humans exposed to the substance. Exposure to the substance may be either direct, due to the presence of the substance in an item or product with which humans come into contact, or indirect, due to the presence of the substance in the environment. One or more of the following types of toxicological effects may result from exposure to a substance: organ- or system-specific (such as cardiovascular or neurological/behavioural); reproductive/ developmental; immunological; carcinogenic; or mutagenic. Effects are considered to be adverse if they result, either directly or indirectly, in functional impairment or pathological lesions that may affect the performance of the whole organism, or which reduce the ability of the organism to respond to an additional challenge.

[1]A useful reference containing reviews of many physical-chemical estimation models may be found in Boethling and Mackay. (2000). Handbook of Property Estimation Method for Chemicals.

In general, the nature, number, severity, incidence and/or prevalence of specific toxicological effects increase with increasing exposure, as determined by the dose, duration and frequency. This is commonly referred to as the dose–response relationship. Besides dose, factors that can influence the toxicological effect include the route of exposure to the substance, species tested (and in the case of animals, strain), genetic susceptibility, physiological state, sex and age of the exposed population.

Toxicological effects may be brief or prolonged, immediate or delayed, reversible or irreversible, single or multiple, nuisance or incapacitating or even life-threatening, and each of these must be considered in assessing the significance of an effect with respect to whether the substance is toxic or is suspected of being toxic. For example, centrilobular necrosis is a more significant toxicological effect than an increased microsomal enzyme activity in liver. A substance shown to induce centrilobular necrosis could, depending on potential human exposure, be found to be toxic while a substance shown to induce only an increase in microsomal enzyme activity could be suspected of being toxic.

Toxicological effects may be classified into two types, threshold or non-threshold, and each of these warrants a different approach to derive the potential risks during assessment.

THRESHOLD EFFECTS

Threshold effects are those that occur only above a certain level of exposure. A toxicological threshold may be defined as a dose below which no adverse effects to the exposed organism will occur. It is postulated that small doses of a harmful substance can be tolerated due to the presence of systems for metabolic detoxification, physiological homeostasis, and cellular adaptation and repair. Below a certain minimum dose, these compensatory mechanisms can mitigate the adverse effects of a substance, even on a continuing basis. At higher dose levels, however, the ability of the organism to compensate or adapt becomes overwhelmed, leading to an impairment in organ function or development of disease state. This may also result following repeated, frequent or continuous exposures to low levels of a substance that can accumulate in the body.

Assessment of substances for which the toxic action has a threshold requires determining the dose–response relationship, with the aim of identifying the highest dose of the substance that does not reveal any adverse effects. This dose is defined as the No-Observed-(Adverse)-Effect-Level (NO(A)EL). This term includes two specific qualifiers: “observed,” which indicates that there may be other effects that have not been detected (e.g. subtle biochemical or specific hormonal effects); and “adverse,” which indicates that not all observed effects are adverse (e.g. a consistent but minor change in body weights or statistically significant changes in serum parameters that are not judged to be toxicologically relevant). If the experimental data do not permit the identification of a NOAEL, the lowest experimental dose at which an adverse effect was observed (Lowest-Observed-Adverse-Effect-Level (LOAEL)) would be identified.

The NOAEL or LOAEL value will depend on the toxicological endpoint being measured. Criteria used to select the most appropriate endpoint for risk assessment (i.e. the critical endpoint) include dose sensitivity, the severity of response, slope of the dose–response curve and whether the effect is reversible or irreversible. Generally, the endpoint that would give the lowest NOAEL or LOAEL would be selected if more than one is available.

Owing to uncertainties in extrapolation of the NOAEL or LOAEL, from experimental studies using animals, to humans (interspecies variability), and because of individual variability in human sensitivity

(intraspecies variability), uncertainty factors must be introduced to obtain a level of exposure below which there would be no or negligible risk for most individuals in the populations of Canada. For long-term human exposures, this level of exposure is often termed the Tolerable Daily Intake (TDI) and represents the amount of substance to which a person can be exposed daily, even over a lifetime, without suffering adverse effects. In order for TDIs to gain formal and widespread acceptance, they would normally be developed, for example, by an international organization and have been the subject of extensive peer review. Extensive external peer review is not feasible within the short timeframes specified for assessment of new substances notified under the NSN Regulations, so the exposure level calculated is considered to be a *provisional tolerable daily intake* (PTDI), representing a dose below which adverse health effects are not expected to occur in human beings.

Ideally, the TDI (or PTDI) is derived from the NOAEL of a chronic study employing the most relevant animal species and the same route of exposure as that expected for humans, or if available, human data. Traditionally, an uncertainty factor of 1 to 10 has been used to account for interspecies extrapolation, and a further uncertainty factor of 1 to 10 has been used to account for variations in human sensitivity. Additional uncertainty factors are used to derive a TDI (or PTDI) when using data from short-term studies to estimate effects resulting from long-term human exposures; when a LOAEL is used instead of a NOAEL; and to accommodate other short-comings in the quality of experimental data. For short-term human exposures, a tolerable acute intake can be derived from short-term animal studies using appropriate uncertainty factors. Further discussion of the use and validation of safety (uncertainty) factors can be found in the report *Biological Safety Factors in Toxicological Risk Assessment* (Health Canada, 1990) and other documents (Kodell and Gaylor, 1999; IGHRC, 2003).

To assess potential risk, the PTDI or tolerable acute intake is compared with the estimated exposure to the substance in the general population and, where appropriate, certain sensitive or high-exposure subgroups. The decision on whether a substance for which the critical effect exhibits a threshold is toxic or suspected to be toxic will depend on whether the estimated exposure exceeds the PTDI or tolerable acute intake while also taking into account the factors discussed in the Guiding Principles for determining health hazards (intrinsic toxicity) and the degree of uncertainty inherent in the type of information (see Figure 1).

NON-THRESHOLD EFFECTS

By definition, non-threshold effects are assumed to occur at any level of exposure to the substance; however, there may well be a "practical" threshold, such as in the case of genotoxic effects, which are a reflection of the interplay between the genotoxicity and the cellular DNA-repair mechanisms. Whether the effect occurs is a function of probability, and although the probability will decrease as the level of exposure decreases, it is assumed that there is no level of exposure for which the probability is zero. In the absence of a plausible alternative mechanism, supported by experimental data, mutagenicity and genotoxic carcinogenicity are currently considered to be non-threshold effects. Assessment of new substances whose toxic action is assumed not to have a threshold requires a careful evaluation of the available information, including results from *in vitro* and *in vivo* biological assays and valid predictive SAR estimates, to determine the weight of evidence of carcinogenicity or human germ cell mutagenicity. New substances will be classified with respect to their potential carcinogenicity and germ cell mutagenicity in accordance with the criteria developed for the assessment of priority substances (Health Canada, 1994).

The approach taken in the assessment of priority substances under CEPA was to designate all substances, for which the critical effect was deemed not to have a threshold, as "toxic" under section 64(c) of the Act

(Hickman, 1991; Health Canada, 1994). An estimate of exposure was not taken into account in establishing whether a substance was toxic; however, exposure was considered in the subsequent review of the need to add the substance to Schedule 1 of the Act and the development of options for control. Since control measures for new substances must be taken before the expiration of the assessment period, it is essential to consider exposure at the same time as the hazard assessment. Consequently, a *de minimus* or "essentially negligible" risk level is established for effects that do not have a threshold.

A new substance that can be classified as "Carcinogenic to Humans" (Group I) or "Probably Carcinogenic to Humans" (Group II) will be considered to be toxic under CEPA if the risk associated with it is not negligible. Similarly, a new substance classified as a "Human Germ Cell Mutagen" (Group I) or a "Probable Human Germ Cell Mutagen" (Group II) will be considered to be toxic if its estimated risk is not negligible.

A substance classified as "Possibly Carcinogenic to Humans" (Group III) or "Possible Human Germ Cell Mutagen" (Group III) or for which the weight of evidence indicates genotoxicity in somatic cells (*in vitro* or *in vivo*) will be suspected of being toxic if the estimated risk associated with it is not negligible.

To determine if the risk associated with a new substance is not negligible and consequently whether any control measures should be taken, it is necessary to obtain a quantitative estimate of the carcinogenic or mutagenic risk where possible. Although minimizing exposure to carcinogenic or mutagenic substances to the greatest extent possible is desirable, control measures to reduce exposure may not be warranted if the incremental risk is sufficiently small that it could be considered essentially negligible.

The risk to humans is usually estimated quantitatively using mathematical models that use data from experimental studies in animals or epidemiological studies to determine concentrations of the substance at which humans may be exposed. Consequently, it may be necessary to determine a level of exposure associated with a negligible level of risk (e.g. a lifetime risk of 1 in 10^6). The range for estimated low-dose cancer risk generally considered by various agencies to be "essentially negligible" lies between 1 in 10^5 and 1 in 10^6 (Health Canada, 1996). In the evaluation of new substances, a value of estimated cancer risk falling within or below this range is not considered to represent a significant risk of carcinogenicity in the general public.

Application of Data from Toxicity Studies and Toxicological Information from Structure Activity Relationships for Determining "Toxic" or "Suspicion of Toxicity"

The application of toxicological data from specific toxicity tests as well as predictive SAR estimates to support a conclusion that a substance is toxic or is suspected of being toxic will be described in this section.

In addition to data from toxicity studies carried out on the notified substance or similar surrogate substances consideration is given during evaluation to toxicity estimates generated using SARs. These estimates fall into two main categories: those based on qualitative structure activity relationships (read-across; surrogate information provided with notifications or obtained from searching the in-house databases) and those based on quantitative structure activity relationships (QSARs). SAR data must be accompanied by supporting information or appropriate validation of the estimate (see Guidelines, section 5.4.2, EC/HWC, 1993) before they can be used in the determination of whether a substance is toxic or suspected of being toxic.

In most cases, estimates of toxicological endpoints using SARs will introduce two levels of uncertainty: firstly, the uncertainty from using an estimated rather than a measured outcome, even if the estimate has a high level of confidence; and secondly, the uncertainty from using data that are derived from toxicological studies on animals. Therefore, the application of valid SAR data will usually be limited to being additional information to support a conclusion that a substance is suspected of being toxic. However, SAR estimates that are based on toxicological data on humans may be used as stronger evidence to support a conclusion that a substance is toxic. Data on humans are mainly limited to skin irritation and skin sensitization studies, but can also be applied if the new substance bears close resemblance to a pharmaceutical or another existing commercial chemical agent with a known human health effects profile.

ACUTE TOXICITY STUDIES

In an acute toxicity study, experimental animals (usually rodents) are administered a single dose of the test substance or multiple doses within 24 hours, observed for a period of at least 14 days, after which the animals are sacrificed. This type of study usually focuses on the potential lethality of the substance with the determination of the LD_{50} or LC_{50}, but with careful cage-side observation and necropsy, more subtle toxicological effects may be noted. With the use of animals of both sexes, sex differences in the response can be detected.

Since acute toxicity studies will often not produce a dose–response relationship with a well-defined NOAEL, or provide information on adverse effects other than lethality, applying additional uncertainty factors to the LD_{50} value may be necessary to derive a tolerable acute intake for short-term human exposures.

Data generated from an acute toxicity test can support the following conclusions:

- The substance is considered to be toxic if short-term human exposure to the substance, for example, through direct exposure, is predicted to exceed the tolerable acute intake.

- The substance is suspected to be toxic if:

  a) the adverse effects observed in the acute toxicity study may reasonably be expected to occur at lower levels of prolonged or repeated exposures and if prolonged or repeated exposures to the substance are plausible. For example, there may be indications of specific organ toxicity, neurological impairment, or potential for accumulation of the substance in the body;

  b) it were to meet the classification of a "very toxic material" under the Controlled Products Regulations of the *Hazardous Products Act*. A substance is included in this classification if one of the following results is obtained in an acute mammalian test:

oral $LD_{50} < 50$ mg/kg,
dermal $LD_{50} < 200$ mg/kg,
inhalation, 4-hour exposure: gas $LC_{50} < 2500$ ppm
vapour $LC_{50} < 1500$ ppm
dust, mist, fume $LC_{50} < 500$ mg/m^3; and

c) the substance is structurally similar to a substance or substances that meet the classification of a very toxic material above and there are no convincing contrary experimental data for the substance.

REPEATED DOSE (14- OR 28-DAY) STUDIES

In a repeated-dose toxicity study, experimental animals (usually rodents) are exposed to the test substance daily for at least 14 or 28 days, then observed for a period of at least 14 days, after which the animals are sacrificed. A repeated-dose study can provide information on the clinical, biochemical, haematological and histopathological effects of the test substance. Delayed effects of the substance that may occur due to accumulation of the substance in the animal or other mechanism can be detected in a repeated-dose study. With the use of animals of both sexes, sex differences in the response can be detected.

A repeated-dose study in animals can provide information on potential adverse effects in humans who may be exposed to the substance over a short period of time and also adverse effects that may reasonably be expected to occur at lower levels during prolonged or repeated exposures. This would be strengthened if there were evidence to suggest the substance may accumulate in the body. The observed effects may also be indicative of adverse effects that may occur following longer exposure to the substance, even though the observed effects themselves are not considered adverse.

Although longer than an acute toxicity study, a 14- or 28-day repeated-dose study is not of sufficient duration to extrapolate confidently to chronic exposures. Consequently, if data from a 14- or 28-day repeated-dose study are to be used to derive a PTDI for longer term exposures, an additional uncertainty factor must be used. An uncertainty factor of up to 100, in addition to the uncertainty factors for intra- and interspecies variation, has been recommended for extrapolations to chronic exposures (IRIS, 1993; I. Chu, 1994). Calculation of a PTDI for subchronic or chronic exposures from a repeated-dose study would be appropriate only if the adverse effects upon which the PTDI is based are reasonably expected to occur following prolonged exposures.

Data generated from a repeated-dose toxicity test can support the following conclusions:

- The substance may be considered to be toxic if the direct short-term human exposure to the substance may exceed the acceptable short-term intake.

- The substance may be suspected of being toxic if:

  a) the long-term human exposure to the substance may exceed the PTDI; and

  b) it is structurally similar to a substance or substances for which any of the above criteria would be met and there is no convincing contrary experimental data on the substance.

SUBCHRONIC (90-DAY) STUDIES

In a subchronic toxicity study, experimental animals (usually rodents) are exposed to the test substance daily for at least 90 days, with an option of satellite groups, then observed for a period of at least 14 days, after which the animals are sacrificed. A subchronic study can provide information on the clinical,

biochemical, haematological and histopathological effects of the test substance that may occur following prolonged and repeated exposures. Delayed effects of the substance that may occur due to accumulation of the substance in the animal or other mechanism can be detected in a subchronic study. With the use of animals of both sexes, sex differences in the response can be detected.

Data generated from a subchronic toxicity study can support the following conclusions:

- The substance may be considered toxic if the potential human exposure to the substance exceeds the PTDI.

- The substance may be suspected of being toxic if:

  a) the observed effects are believed to be indicative of adverse effects that may occur following longer exposure to the substance even though the observed effects themselves are not considered adverse and the potential exposure exceeds the PTDI. This conclusion would be strengthened if there was evidence to suggest the substance may accumulate in the body; or

  b) the substance is structurally similar to a substance or substances for which any of the above criteria would be met and there is no convincing contrary experimental data on the substance.

CHRONIC TOXICITY

In the absence of chronic toxicity studies (not required under NSN Regulations and usually not available for most new substances), QSAR methodology is used for estimating the chronic LOAEL (TOPKAT; HDI, Accelrys software). Where possible, available data for closely similar substances are considered during assessment.

- the substance may be suspected to be toxic if a positive QSAR estimate of high confidence can be made and the potential exposure exceeds the PTDI. There should be no convincing contrary experimental data on the substance, such as from a subchronic toxicity study available for the substance or closely matching analogue.

DEVELOPMENTAL TOXICITY

In the absence of developmental toxicity studies (not required under NSN Regulations and usually not available for most new substances), QSAR methodology is used for estimating this endpoint (TOPKAT; HDI, Accelrys software). Where possible, available data for closely similar substances are considered during assessment.

- the substance may be suspected to be toxic if a positive QSAR estimate of high confidence can be made and the potential exposure exceeds the PTDI. There should be no convincing contrary experimental data on the substance.

GENOTOXICITY

In the absence of a plausible alternative mechanism supported by experimental data, mutagenicity is considered to be a non-threshold effect. Consequently, it is assumed that no level of exposure to a mutagenic substance is without some probability that a mutation will occur. At present, it is not possible to quantify levels of risk for mutagenic responses. Under the New Substances Program, the substances are treated as if they were carcinogens. This approach is considered to provide a conservative estimate of the risk; however, until methods are available to calculate genotoxic risks, this cannot be confirmed. This practice was adopted following discussions with the staff in the new chemicals program of the U.S. EPA in 1995.

To estimate the postulated carcinogenic risk associated with a given exposure level, the carcinogenic potencies of comparable chemicals are used. A database incorporating information from the Gold database that can be searched by chemical structure or substructures has been developed in-house for this purpose. If no information is available for structurally similar chemicals, a potency value is selected from the distribution of potencies of all chemicals published in the Gold database (Gold et al., 1995). The potency value is selected on a case-by-case basis (e.g. the median value could be used, or a less potent value chosen if the substance is a weak mutagen).

The determination of whether a mutagenic substance is toxic or is suspected of being toxic will depend on the weight of evidence to support the conclusion that the substance is mutagenic, and whether the mutation occurs in the germ cells or in somatic tissue. The distinction between germ cell and somatic cell mutations is made to reflect the relative "closeness" of the mutation to the ultimate disease state. A germ cell mutation is sufficient to induce an adverse effect (i.e. a heritable mutation), whereas a somatic cell mutation indicates only the potential for carcinogenicity or germ cell mutagenicity.

Factors contributing to the weight of evidence of mutagenicity include the number and type of mutagenicity tests performed (e.g. *in vitro* or *in vivo*), whether the genetic endpoint investigated presents a bona fide genetic consequence (e.g. gene mutation, chromosomal aberrations including micronuclei) or is an indicator of possible genetic effects (e.g. sister chromatid exchange, DNA adducts), and the structural similarity of the substance to known mutagenic substances.

Data generated from genotoxicity tests can support the following conclusions:

- The substance may be considered to be toxic if it meets the criteria for Group I or II of the "Criteria for Classification of Mutagenicity in Germ Cells" and the associated risk is not negligible.

- The substance may be suspected to be toxic if:

  a) it meets the criteria for Group III of the "Criteria for Classification of Mutagenicity in Germ Cells" and the associated risk is not negligible. For new substances, the most relevant criterion in Group III is expected to be positive results from *in vivo* mammalian studies for gene mutation or chromosomal aberrations (including micronuclei), when there is no convincing pharmacokinetic, metabolic or other data to suggest that humans would not be subject to the genotoxic effects;

  b) it has been found to be positive in an *in vivo* mammalian indicator test for mutagenicity and the associated risk is not negligible. There should be no convincing conflicting data from *in vivo* mammalian tests for gene mutation or chromosomal aberrations (including micronuclei). Indicators include tests for sister chromatid exchange, genetic recombination, DNA adducts and induction of DNA repair;

c) it has been found to be positive in an *in vitro* test for gene mutation or chromosomal aberrations (including micronuclei) and the associated risk is not negligible. There should be no convincing conflicting data from *in vivo* mammalian tests for the same genetic endpoint that gave a positive response in the *in vitro* test;

d) it has been found to be positive in an *in vitro* indicator test for mutagenicity and the associated risk is not negligible. There should be no convincing conflicting data from *in vitro* or *in vivo* mammalian tests for gene mutation, chromosomal aberrations or micronuclei; or

e) it is structurally similar to a substance or substances for which any of the above criteria would be met and there is no convincing contrary experimental data on the substance.

Conflicts between results from *in vivo* and *in vitro* tests or between tests for different genetic endpoints must be considered on a case-by-case basis.

CARCINOGENICITY

Both qualitative and quantitative methods are available for estimating carcinogenicity using SARs. Included among the qualitative SAR is a consideration of structural alerts by visual pattern recognition aided by substructural searches conducted within carcinogenicity databases, such as the ISIS-based GOLD database that has been developed from the sixth plot of the GOLD Carcinogenic Potency Database. This database comprised information gleaned from the NTP/NCI carcinogenicity studies as well as from the published literature on more than 1,200 compounds (Gold et al., 1995). The ISIS-based GOLD database, in use, allows one to carry out substructural searches on chemical fragments of interest that are present in a notified substance. The availability of predictive software in conjunction with this searchable form of the GOLD database provides an evaluation procedure that provides for a much larger scope than the use of predictive software alone.

For quantitative SAR predictive estimates, TOPKAT (currently distributed by Accelrys software) is used. This software uses an approach that is different from the functional group pattern matching procedure that is adopted by simpler first generation toxicity prediction software (such as Hazardexpert; Compudrug USA). TOPKAT uses a high quality training set of compounds to derive a mathematical construct that incorporates electrotopological state values (numerical quantifiers of molecular structure describing the electron content, environment of an atom and a group of atoms in a molecule, as well as numerical descriptors of molecular shape [up to 7 orders], symmetry and bulk). A consideration of these parameters takes into account the environment in which a functional group and smaller structural elements in a molecule exist and react with other molecules. It must be noted that when using these tools, each notified chemical is examined and judged on a case-by-case basis, and proper validation is carried out prior to accepting an estimated or predicted result. During the conduct of such predictive estimates, some of the possibilities that might arise are described below:

- The substance may be suspected to be toxic if a positive QSAR or SAR estimate of high or moderate-high confidence can be made and the associated risk is not negligible. There should be no convincing contrary experimental data on the substance.

- The substance may be suspected to be toxic if available read-across information from suitably justified structural analogues indicates that these are carcinogenic.

The ISIS-based GOLD database serves as an important tool in developing risk management strategies by helping to identify compounds that closely match the notified substance. The TD_{50} values for these similar substances can be used in conjunction with the computed exposure values for deriving the estimated health risk based on lifetime exposure scenarios.

SKIN IRRITATION

In a skin irritation test, the substance is applied dermally to the experimental animal, usually for a period of 4 hours, and the skin is examined for erythema and oedema 60 minutes, 24, 48 and 72 hours following exposure. The grading of the responses is subjective and ranges from no response to a severe response.

Data generated from a skin irritation study can support the following conclusions:

- The substance may be considered toxic if it has been shown to be corrosive or a severe irritant, or is expected to be corrosive or a severe irritant based on considerations of pH and chemical reactivity (including labelling requirements imposed by other Acts and Regulations, such as the *Hazardous Products* Act and the Consumer Chemicals and Containers Regulations), and humans may be directly exposed to comparable concentrations of the substance; and

- The substance would not be suspected of being toxic if based on test data for the substance or well-justified structural analogues or considerations of pH and chemical reactivity, the observed or estimated irritation is of moderate intensity, and is reversible.

SKIN SENSITIZATION

In a skin sensitization study, the substance is applied to the test animals by intradermal injection and/or epidermal application, and following a rest period (no exposure) of usually 10 to 14 days, the test animals are again exposed to the substance. The skin at the challenge site is examined 3, 24 and 72 hours following this challenge exposure. The grading of the responses is subjective, ranging from no response to strong, and is also based on the proportion of animals affected.

Data generated from a skin sensitization study can support the following conclusions:

- The substance may be considered to be toxic or capable of becoming toxic if it has been shown to be a strong sensitizer in animals or human studies and humans may be directly exposed to the substance.

- The substance may be suspected of being toxic if:

  a) read-across test information for structural analogues or SAR/QSAR estimates indicate that it might be a strong sensitizer; and

  b) test data in animals or human volunteers for the substance or a structurally similar substance or suitably validated SAR/QSAR estimates indicate that the substance might be a moderate sensitizer.

Endnote

This document describes the approaches adopted by the New Substances Program, Health Canada, during the first 10 years of the implementation of the New Substances Notification provisions and Regulations (since July 1, 1994). Following the renewal of CEPA (1999), there were some changes to the New Substance Provisions, notably the Significant New Activity Notice. These and other changes within CEPA, 1999, as well as those arising out of the amended NSN Regulations (implemented in 2005) do not alter appreciably the basis for determining suspicion of toxicity for new substances.

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