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Evaluation of inhalation TTC values with the database RepDose

S.E. Escher [a,*], I. Tluczkiewicz [a], M. Batke [a], A. Bitsch [a], C. Melber [a], E.D. Kroese [b], H.E. Buist [b], I. Mangelsdorf [a]

[a] Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Str. 1, 30625 Hannover, Germany
[b] Department of Research and Development, TNO Quality of Life, Utrechtseweg 48, 3704 HE Zeist, The Netherlands

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ABSTRACT

The thresholds of toxicological concern (TTCs) define limit values for substances of unknown toxicity below which dietary intake is considered to be of no concern to human health. The TTC concept has already been used for risk assessment of e.g. food contaminants or flavoring substances and is in discussion to be applied to other classes of compounds such as cosmetic ingredients, household products, non-relevant metabolites in drinking water, and impurities in pharmaceuticals. The present publication aimed to evaluate whether the current TTC concept can also be applied to define limit values for inhalation exposure, using a data set of 203 industrial chemicals from the database RepDose.

It has been shown, that the NOEC values in classes 1, 2, and 3 are distributed over six orders of magnitude resulting in a considerable overlap between the distribution curves for the three classes. Inhalation thresholds for Cramer classes 1 (compounds likely to be of low-toxicity), 2 (compounds likely to be of moderate toxicity), and 3 (compounds suspect for high toxicity) were analyzed close to the approach described by Munro for oral TTCs. The 5th percentiles NOEC of Cramer classes 1–3 result in thresholds of 1.5×10^{-3} ppm for Cramer class 1 and 2.2×10^{-5} ppm for Cramer class 3. A threshold could not be derived for class 2 because of the small number of compounds available. If calculated as body doses, the inhalation thresholds for classes 1 and 3 (71 and 4 μg/person/d, respectively) are considerably lower than the oral thresholds derived by Munro (1800 and 90 μg/person/d). It has been shown that one reason for this difference is the high sensitivity of the respiratory tract to local effects.

In a next step, the values obtained were further refined. If organophosphates or compounds with structural alerts for genotoxicity are excluded, the TTC in Cramer class 1 increases, whereas the TTC in Cramer class 3 remains the same. Based on these analyses two inhalation TTCs for non-genotoxic compounds are proposed: 3.6×10^{-3} ppm (180 μg/person/d) for Cramer class 1 and 2.4×10^{-5} ppm (4 μg/person/d) for Cramer class 3.

1. Introduction

Thresholds of toxicological concern (TTCs) have been developed for risk assessment of compounds of known chemical structure for which no compound-specific toxicity data are available (Munro et al., 1996). Below the TTC value the risk to human health is assumed to be negligible. The TTC may be used as a substitute for substance-specific information in situations where there is limited or no information on the toxicity of a compound, and where human exposure is so low, i.e. below the corresponding TTC, that adverse effects are not to be expected.

In 2004, Kroes et al. proposed a detailed decision tree to identify the appropriate TTC value for an untested substance based on its structural features (Kroes et al., 2004). With this approach first "cancer" thresholds were assigned to certain groups of substances. For compounds with structural alerts for genotoxicity a TTC value of 0.15 μg/person/d was derived, and for non-genotoxic substances on which no further information is available a general TTC value of 1.5 μg/person/d was proposed. The general threshold was extrapolated from TD50 values in a database of more than 700 carcinogenic substances, for which a risk of 1 to 10^6 is assumed to be acceptable (Cheeseman et al., 1999). Recently, Felter et al. (2009) described the inclusion of data on genotoxicity like the AMES test to refine the very low TTC of 0.15 μg/person/d for substances with structural alerts for genotoxicity. Only few structural classes of highly toxic chemicals were identified not to be covered by the current TTC approach, e.g. steroids, polyhalogenated dibenzo-*p*-dioxins, polyhalogenated biphenyls/dibenzofurans, aflatoxin-like, *N*-nitroso-, or azoxy-compounds.

Abbreviations: LO(A)EL, lowest observed (adverse) effect level; NO(A)EL, no observed (adverse) effect level; N(L)OEC, no (lowest) observed effect concentration.

* Corresponding author. Fax: +49 511 5350 335.
E-mail address: Sylvia.Escher@item.fraunhofer.de (S.E. Escher).

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For organophosphates (OPs) a special TTC value of 18 μg/person/d has been derived (Munro et al., 2008).

According to the Kroes decision tree, the non-genotoxic substances are then grouped into three broad structural classes using the Cramer decision tree (Cramer et al., 1978). Cramer class 1 contains "innocuous" structures for which metabolism and mode of action data suggest low-toxicity. Cramer class 2 contains less innocuous structures, and Cramer class 3 focuses on structures which can be assumed to be toxic.

In 1996, Munro and coworkers used a probabilistic approach to derive threshold values for each of the three Cramer classes. Munro used a database containing food substances, pharmaceuticals, industrial, environmental, agricultural, and consumer chemicals which had been tested in subacute to chronic repeated-dose toxicity studies with oral dosage. The TTCs for Cramer classes 1–3 are based on the 5th percentile NOAEL in each class, divided by an assessment factor of 100, and multiplied by an average human body weight of 60 kg to derive thresholds of 1800, 540, and 90 μg/person/d for Cramer classes 1, 2, and 3, respectively. This conservative approach gives a 95% probability that the risk for an untested substance is negligible, if the estimated intake does not exceed the TTC value. The database initially used by Munro to derive the above described TTCs for Cramer classes 1–3 included substances with structural alerts for genotoxicity as well as organophosphates. A recent publication stated that the very conservative TTC of Cramer class 3 was dominated by the NOAELs for OPs and organohalogens (Munro et al., 2008). Excluding OPs increases the threshold for Cramer class 3 to 180 μg/person/d, excluding both OPs and organohalogens raises it to 600 μg/person/d. The exclusion of "toxic" compounds from Cramer class 3 leads to a threshold which is comparable to that of Cramer class 2 (540 μg/person/d). Thus it has to be discussed what kind of thresholds will be the goal of TTC refinement: TTCs for specific categories of substances like for organophosphates and/or organohalogens, or generally applicable thresholds for structural classes defined as toxic, moderately toxic, and non-toxic according to Cramer, or an alternative method.

Up to now, the TTC values have been used for assessment of food contact materials by the US Food and Drug Administration (US FDA) and of flavoring substances by the European Food Safety Authority (EFSA), as well as for impurities in pharmaceuticals (Barlow, 2005; Cheeseman et al., 1999; Delaney, 2007; Kroes et al., 2000, 2004; Müller et al., 2006; Munro et al., 1998, 2008; Renwick, 2004). These applications are all characterized by very low exposure levels. Currently, it is under discussion whether the TTC can also be applied to the risk assessment of a broader variety of substances. Its use has been proposed for personal and household care products (Blackburn et al., 2005), cosmetic ingredients (Kroes et al., 2007), non-relevant metabolites from pesticides in drinking water (Melching-Kollmuß et al., 2010), and plant extracts (Re et al., 2009). Furthermore, inhalation TTCs have been derived for ingredients in consumer products (Carthew et al., 2009).

Inhalation is an important route of exposure for consumers (e.g. indoor air) and in the occupational context. Relevant *in vivo* data are, however, often not available for many compounds which are typically found in such environments. Therefore, it is of great interest to develop the TTC concept further and to derive inhalation-specific threshold values. We used the database RepDose that contains mainly existing chemicals (Bitsch et al., 2006, www.fraunhofer-repdose.de) to derive inhalation thresholds for Cramer classes 1–3 in a way close to the approach described by Munro et al. (1996) for oral TTCs. General inhalation thresholds for Cramer classes 1–3 were described, based on all available chemicals in RepDose which have been subject to inhalation studies. The 5th percentiles of NOEC values in ppm or mg/m^3 were used to determine inhalation TTCs. Furthermore, we evaluated how local and systemic toxicity influence the derived thresholds values. Local and systemic NOEC values were distinguished and target organs which occur at study LOEC were analyzed. Kroes et al. (2004) proposed to assign particular thresholds to genotoxic substances or organophosphates and exclude these from Cramer classes 1 to 3. The same procedure has been applied to the data set used to derive inhalation thresholds. Both groups, organophosphates and substances having structural alerts for genotoxicity, were excluded and thresholds were derived for the remaining substances in Cramer classes 1–3.

2. Materials and methods

2.1. Analysis of data and derivation of TTCs

The database RepDose (www.fraunhofer-repdose.de), developed at the Fraunhofer ITEM, has been used for the analyses. RepDose is a continuously growing database (Bitsch et al., 2006). For this report, the status of November 2009 was used. Currently, it contains over 650 mainly industrial chemicals and also some pesticides tested in repeated-dose toxicity studies with oral (gavage, diet, and drinking water) and inhalation exposure of rats and mice.

The classification of chemicals into Cramer classes was performed with the open source program Toxtree (http://ambit.acad.bg/toxtree).

As RepDose contains more than one study per chemical we prioritized the relevance of studies according to exposure duration. First, only studies with chronic exposure duration ($\geqslant$700 days) were considered. For substances which have not been investigated in a chronic study, subchronic (84–98 days) and then subacute (21–32 days) studies were used for the analysis. Whenever more than one study in one exposure category was available the study with the lowest NOEC value was analyzed. A total of 203 compounds were identified and analyzed (Appendix A). For values derived from short-term studies extrapolation factors of 2/6 for the corresponding subchronic/subacute studies were applied (ECHA, 2008). If a NOEC could not be identified by the procedure described above, it was extrapolated from LOEC to NOEC by applying a factor of 3 according to ECHA (2008).

Both ppm and mg/m^3 were used as dose measures for inhalation studies. If not provided in the individual studies, Eq. (1) was used to convert ppm to mg/m^3 and vice versa, as most of the RepDose compounds are vapors. The ppm values allow comparison of NOECs on a molar basis, whereas mg/m^3 have been used for calculation of body doses and derivation of threshold values that could be directly compared to TTCs derived from the oral route.

The geometric means, geometric standard deviations, medians, 5th and 95th percentiles of the distribution of the NOECs in the three Cramer classes were derived. Statistical analysis of the data was performed using the program STATISTICA from Statsoft.

TTC values were derived based on the 5th percentile NOEC as described by Munro et al. (1996) for the oral route. Thresholds for concentrations in air were calculated from the 5th percentile NOEC (ppm and mg/m^3) of Cramer classes 1–3 using Eq. (2). Furthermore, thresholds for daily exposure of humans (μg/person/d) were calculated from the NOEC in mg/m^3 using Eqs (3) and (4) to allow direct comparison to the corresponding values derived by Munro and coworkers for oral exposure.

Most animal studies use exposure durations of 6 h/d on 5 d/week. To account for exposure of consumers the 5th percentile was normalized to a daily exposure of 24 h and 7 days exposure per week (d_{exp}). Subsequently, a safety factor of 25 was used, which consists of an interindividual factor of 10 for interindividual

variation in humans and a factor of 2.5 for remaining uncertainty for interspecies differences in toxicodynamics (Renwick, 1993; ECHA, 2008).

Default values used in the calculation are:

- Average human body weight of 60 kg (Munro et al., 1996).
- Standard human respiratory volume ($V_{resp.human}$): 20 m^3 for consumers (ECHA, 2008).

Eq. (1): Conversion of NOECs: mg/m^3 to ppm.

$$\text{NOEC}\ (\text{mg/m}^3) = \frac{\text{NOEC}\ (\text{ppm}) \times \text{MW}\left(\frac{\text{g}}{\text{mol}}\right)}{24.45\left(\frac{\text{l}}{\text{mol}}\right)} \quad (1)$$

Eq. (2): TTC for concentrations in ppm and mg/m^3.

$$\text{Threshold} = \frac{5\text{th}_{\text{percentile}}\text{NOEC} \times \text{d}_{\text{exp}}}{10 \times 2.5} \quad (2)$$

Eq. (3): Conversion of NOEC values in mg/m^3 to body doses.

$$\text{NOEL}\ (\mu\text{g/kg/d}) = 5\text{th}_{\text{percentile}}\text{NOEC}\left(\frac{\text{mg}}{\text{m}^3}\right) \times \text{d}_{\text{exp}} \times \left(\frac{V_{\text{resp.human}}\left(\frac{\text{m}^3}{\text{d}}\right)}{\text{bw}_{\text{human}}\ (\text{kg})}\right) \times 1000 \quad (3)$$

Eq. (4): Derivation of TTC values in µg/person/d.

$$\text{Threshold}\ (\mu\text{g/person/d}) = \frac{\text{NOEL}\ (\mu\text{g/kgbw/d}) \times \text{bw}_{\text{human}}\ (\text{kg})}{10 \times 2.5} \quad (4)$$

MW, molecular weight (substance-specific); d_{exp}, daily exposure (6/24 h × 5/7 d); $V_{resp.human}$, human respiratory volume; bw_{human}, human body weight; 5th NOEC = 5th percentile of NOEC distribution.

2.2. Distinction between local and systemic NOEC values

To evaluate whether local and systemic threshold values have to be distinguished, thresholds were analyzed for "locally acting" and "systemically acting" substances. Locally acting substances were defined as those showing effects predominantly in local target organs, i.e. organs of first contact, where ADME processes have not yet occurred. About 60 target organs are documented in RepDose. Eye and organs of the respiratory tract such as nose, larynx, pharynx, trachea, lung, and bronchi were defined as "local" target organs. All other organs such as spleen, testes, liver, kidney, etc. were classified as "systemic" target organs. Based on the affected target organs "local" NOEC/LOEC values and "systemic" NOEC/LOEC values were derived for each chemical in both subsets. NOEC values were standardized using assessment factors for time and LOEC to NOEC extrapolation as described above.

2.3. Identification of genotoxic compounds

Several tools are available that indicate the genotoxic potential of substances using e.g. structural alerts. In our approach, we used the open source software Toxtree (http://ambit.acad.bg/toxtree) to classify substances as putatively genotoxic or non-genotoxic.

3. Results

3.1. Derivation of inhalation TTCs

A total of 203 chemicals from the RepDose database were analyzed to derive TTCs for inhalation exposure. Application of the Cramer decision tree gave the following results (see also Table 1): most of the chemicals (138, corresponding to 68%) are classified as potentially toxic (class 3), 7 (3%) as potentially moderately toxic (class 2), and 58 (29%) as potentially of low-toxicity (class 1). The grouping of chemicals derived for the RepDose data set is similar to the grouping obtained for the database used by Munro to derive oral thresholds for Cramer classes 1–3 (Munro et al., 1996). In the Munro database 73% of the analyzed compounds were grouped into class 3 (446 out of 611), 5% into class 2, and about 22% into class 1.

Fig. 1 illustrates the distribution of NOEC values observed in the 203 inhalation studies analyzed for TTC derivation. It can be noticed that the NOEC values in our data set cover a wide range from 0.001 to 100,000 ppm. Only a small number of chemicals though have very low NOEC values below 0.1 ppm (19%) or very high NOEC values above 100 ppm (15%), whereas the majority of the analyzed compounds (64%) lie in between. It could be controversially discussed which NOEC limit values have to be used to classify compounds as toxic, moderately toxic, or of low-toxicity after inhalation exposure. We decided to compare the Cramer classes of our data set with the limit values of the GHS classification

Table 1
TTCs for inhalation exposure; NOEC values were analyzed using the units ppm and mg/m^3.

Dataset from RepDose	Type	Cramer class	*N* (*N*%)	NOEC (ppm)					TTC		
				GM	GSD	Median	5th	95th	ppm[(*)]	mg/m^3[(*)]	µg/person/d
All chemicals (*N* = 203)	General	1	58 (29%)	13.7	11.7	23.6	2.1×10^{-1}	338	1.5×10^{-3}	3.6×10^{-3}	71
		2	7 (3%)	0.7	8.8	0.9	2.8×10^{-2}	14	2.0×10^{-4}	4.8×10^{-4}	10
		3	138 (68%)	1.7	62.1	1.0	3.1×10^{-3}	5002	2.2×10^{-5}	1.8×10^{-4}	4
Chemicals with local targets (N = 102)	Local	1	26 (25%)	10.6	15.6	9.4	2.1×10^{-2}	500	1.5×10^{-4}	6.1×10^{-4}	12
		2	6 (6%)	0.6	10.2	0.8	2.8×10^{-2}	13	2.0×10^{-4}	4.8×10^{-4}	10
		3	70 (69%)	0.8	47.6	0.5	3.3×10^{-3}	1996	2.4×10^{-5}	1.9×10^{-4}	4
	Systemic	1	26 (25%)	19.7	8.3	29.3	2.5×10^{-1}	254	1.8×10^{-3}	8.9×10^{-3}	179
		2	6 (6%)	3.3	8.1	1.6	5.2×10^{-1}	51	3.7×10^{-3}	1.1×10^{-2}	214
		3	70 (69%)	1.2	27.8	1.0	7.0×10^{-3}	124	5.0×10^{-5}	3.2×10^{-4}	6
Chemicals with only systemic targets (*N* = 97)	Systemic	1	29 (30%)	22.5	10.7	41.2	3.4×10^{-1}	3389	2.4×10^{-3}	4.8×10^{-3}	95
		2	1								
		3	67 (69%)	4.7	89.6	4.2	6.0×10^{-3}	19938	4.3×10^{-5}	3.9×10^{-4}	8
All chemicals with systemic targets (*N* = 199)	Systemic	1	55 (28%)	21.1	9.3	33.5	2.5×10^{-1}	338	1.8×10^{-3}	4.8×10^{-3}	95
		2	7 (3%)	3.1	6.8	2.0	5.2×10^{-1}	51	3.7×10^{-3}	1.1×10^{-2}	214
		3	137 (69%)	2.3	54.0	1.7	6.0×10^{-3}	5002	4.3×10^{-5}	3.2×10^{-4}	6

GM, geometric mean; GSD, geometric standard deviation; NOEC, no observed effect concentration; TTC, threshold of toxicological concern.
[*] *Exposure:* 24 h/d, 7 d/week.

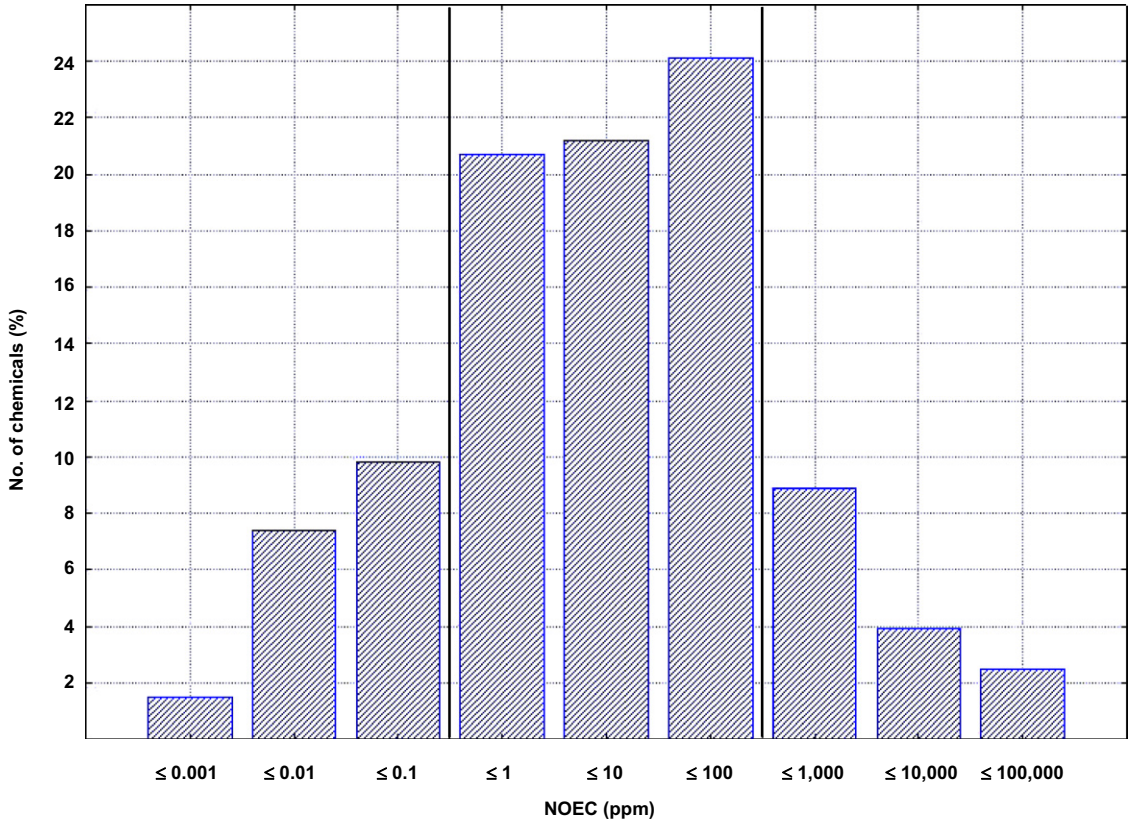

Fig. 1. NOEC distribution of the 203 inhalation studies in RepDose.

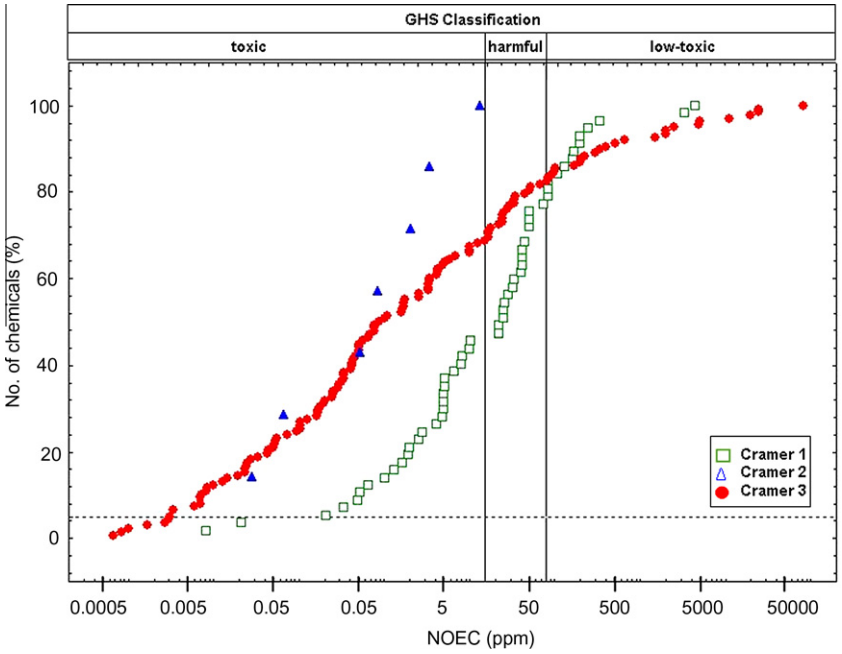

Fig. 2. Cumulative distribution of NOECs of chemicals in RepDose in the 3 Cramer classes. Cramer classes 1 (green squares), 2 (blue triangles), and 3 (red circles). The 5th percentile, which defines the TTCs is indicated as dotted line. The GHS cut-off NOECs of 15 and 80 ppm are indicated (black line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Globally Harmonized System of Classification and Labeling of Chemicals; GHS, 2007) for repeated-dose toxicity. Fig. 2 depicts the cumulative distribution of NOEC values in Cramer classes 1–3 and the inhalation limits of the GHS classification (toxic $\leqslant$15 ppm, harmful >15 and $\leqslant$80 ppm, low-toxicity >80 ppm). These limit values were obtained by dividing the LOECs originally

provided by GHS by 3 to extrapolate from LOECs to NOECs and thus to allow comparison with the NOECs shown here (GHS, 2007). Both GHS and Cramer classify the majority of compounds as toxic; however, comparing the distribution of NOECs in Cramer classes 3 and 1 to the classification given by GHS (toxic and of low-toxicity) it can be stated that 47% of all substances can be considered as toxic in Cramer class 1 and 31% as harmful or of low-toxicity in Cramer class 3. It can thus be stated that the Cramer and GHS classifications disagree on the grouping of chemicals for many compounds of our data set.

In 1978, Cramer et al. developed a decision tree to predict the toxicity of compounds based on their structural properties. The majority of compounds in Cramer class 1 (green curve) have NOEC values between 0.1 and 10,000 ppm. Cramer class 3 (red curve) covers values from 0.001 to 100,000 ppm. The geometric means of the NOECs of the chemicals in Cramer classes 1, 2, and 3 are 13.7, 0.7, and 1.7 ppm, respectively (Table 1). The wide spread of values in both classes results in a remarkable overlap between the two classes of low-toxicity and toxic substances.

Following the approach of Munro et al. (1996), who calculated TTCs for the oral route, TTCs for inhalation exposure were to be derived based on the 5th percentiles of the NOECs of the chemicals in RepDose in the three Cramer classes. The 5th percentiles as indicated as dotted line in Fig. 2 are 2.1×10^{-1} ppm for Cramer class 1, 2.8×10^{-2} ppm for Cramer class 2, and 3.1×10^{-3} ppm for Cramer class 3 (Table 1). Although the NOEC values for Cramer classes 1–3 overlap to some extent, the 5th percentiles for Cramer classes 1–3 each differ by a factor of about ten, thus resulting in three clearly discriminated threshold values. The threshold values derived are termed "general" TTCs in the following. On a molar basis, the TTCs calculated for 24 h exposure on 7 d/week of a consumer are 1.5×10^{-3} ppm for Cramer class 1, 2.0×10^{-4} ppm for Cramer class 2, and 2.2×10^{-5} ppm for Cramer class 3. The corresponding TTC values for consumers in mg/m^3 are 3.6×10^{-3}, 4.8×10^{-4}, and 1.8×10^{-4} for Cramer classes 1, 2, and 3, respectively. Only seven chemicals, however, were available for deriving threshold values for Cramer class 2, therefore these TTCs cannot be considered reliable.

The 5th percentiles for classes 1–3 in mg/m^3 were used to derive TTCs as body doses to allow a direct comparison with the TTCs derived for the oral route. The threshold for systemic doses from inhalation is 71 μg/person/d for class 1, 10 μg/person/d for class 2, and 4 μg/person/d for class 3 (Table 1). The values derived by Munro were 1800, 540, and 90 μg/person/d, respectively. Thus, all values derived for inhalation are considerably lower than for the oral route.

3.2. Comparison of local and systemic effects

We further evaluated whether the low TTC values derived for inhalation exposure are due to a special sensitivity of the respiratory tract to local effects. The chemical itself may be more toxic in the respiratory tract, e.g. because of its irritating properties or differences in metabolism/bioactivation in the lung compared to the liver, which may lead to higher toxicity in the lung.

In a first step, the route-dependent sensitivity of the target organs was explored by analyzing the frequency of the target organs that determine the lowest observed effect concentration (study LOEC) in Cramer classes 1 and 3 after inhalation exposure (Fig. 3).

In Cramer class 1, the predominantly affected target organs are nose (15%) and clinical symptoms (14%), followed by kidney (10%), body weight (9%), hematology/clinical chemistry (8%), testes (6%), and liver (4%). In Cramer class 3, the most frequently affected organs in descending order are nose (11%), liver (9%), hematology/body weight/clinical symptoms (8%), clinical chemistry/lung (7%), and kidney (6%).

It can be noticed for Cramer class 1 and to a lesser extent for Cramer class 3 that classical systemic targets such as liver or kidney are affected less frequently at study LOEC compared to local target organs such as nose or lung.

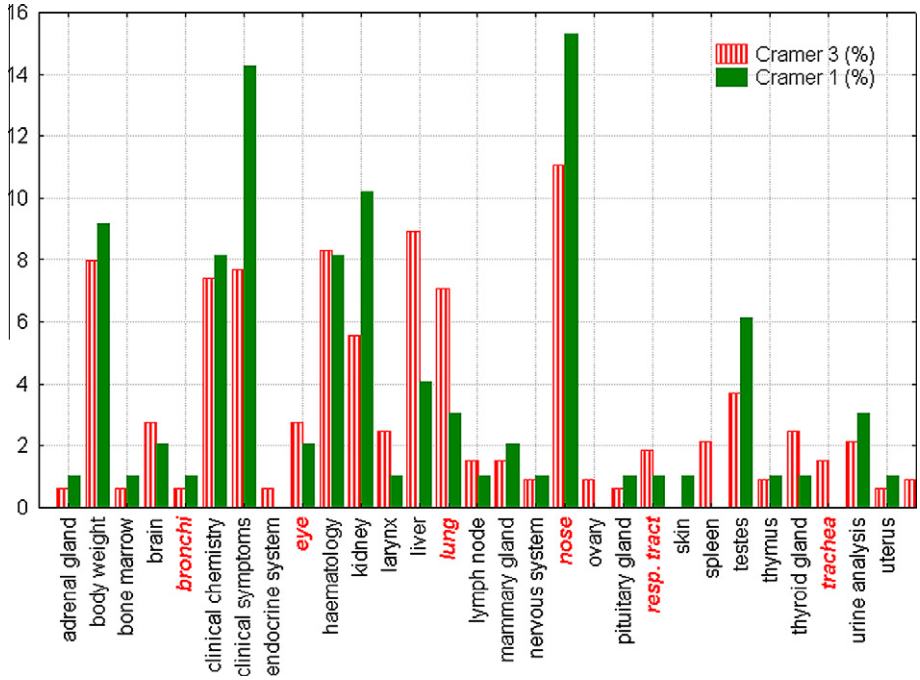

Fig. 3. Target organs that trigger the study LOEC in inhalation studies of Cramer classes 1 (green, filled) and 3 (red, stripped). Local target organs are indicated bold red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Our analysis indicates that for Cramer classes 1 and 3 local effects in the respiratory tract indeed play a major role in determining the LOEC, which may trigger the observed low threshold values (Table 1).

In a second step, we analyzed whether NOECs differ between local and systemic effects/toxicity. For this purpose, the 203 substances in our data set were subgrouped into chemicals with local targets ($N = 102$) and chemicals with only systemic targets ($N = 97$, Table 1). Four chemicals which did not cause any effects in the corresponding studies were excluded from this analysis.

For the subset of 102 chemicals with local effects, a local and a systemic NOEC value were distinguished based on the affected organs. In this data set, 25% of the substances belong to Cramer class 1, 6% to class 2, and 69% to class 3 (Table 1), reflecting the situation in the complete data set. Again, no conclusion can be drawn for Cramer class 2, as it consists of six data points only.

In Cramer class 1, the 5th percentiles and therefore TTCs derived from local NOEC values are lower compared to the systemic values, with a local TTC of 1.5×10^{-4} ppm (6.1×10^{-4} mg/m^3) compared to a systemic TTC of 1.8×10^{-3} ppm (8.9×10^{-3} mg/m^3). In Cramer class 3, the local threshold does not differ significantly from the systemic threshold, being 2.4×10^{-5} ppm (1.9×10^{-4} mg/m^3) and 5.0×10^{-5} ppm (3.2×10^{-4} mg/m^3), respectively.

It has to be considered that the data sets used to derive the above described thresholds are relatively small, so that the 5th percentiles are determined by only few substances of high toxicity (1 substance in class 1 and 3–4 substances in class 3). A trend in the distribution of NOECs to lower or higher values is better characterized by the geometric mean and/or median value of the data set. For Cramer class 1, both values are lower for "local" toxicity than "systemic" toxicity, with the "general" values lying in between. For Cramer class 3, this trend is less evident, as all values are very low.

Furthermore, we analyzed whether there are any particular structural features that account for large differences between systemic and local NOEC values in inhalation studies. Nineteen chemicals of this data set were identified for which the $NOEC_{systemic}$ to $NOEC_{local}$ ratio is higher or equal to 9 (Appendix A).The most frequently occurring structural class is that of carboxylic esters ($N = 7$, 37%). Further structural elements identified in this context were e.g. α,β unsaturated carbonyls ($N = 4$, 21%), aliphatic ethers ($N = 2$, 11%), acyl halogenides ($N = 2$, 11%), diisocyanates ($N = 2$, 11%), diketones ($N = 2$, 11%), and secondary amines ($N = 2$, 11%).

In addition, the NOEC distributions for classes 1–3 of the 97 substances with effects only in "systemic" targets were analyzed and 5th percentiles derived (Table 1). In this data set, the systemic NOEC corresponds to the study NOEC.

With 2.4×10^{-3} ppm (4.8×10^{-3} mg/m^3) the systemic TTC for Cramer class 1 is higher compared to the local TTCs and slightly higher than the general TTC. Again, class 1 is restricted to 29 substances, so that the 5th percentile is triggered by one substance only. But also the median and geometric means in class 1 are higher compared to the values for the general and local thresholds.

With 4.3×10^{-5} ppm (3.9×10^{-4} mg/m^3) the systemic TTC for Cramer class 3 in this data set does not differ from the general and local values. Also the median and geometric means are in the same range as for the general and local data sets in this class, so that a trend to lower thresholds for local toxicity is less evident. As already seen in Fig. 3, systemic target organs are also affected at study LOEC, indicating that low NOEC values are caused by either local and/or systemic toxicity.

Taking together all substances that induce systemic toxicity ($N = 199$), the threshold for class 1 remains at 1.8×10^{-3} ppm (4.8×10^{-3} mg/m^3) and at 4.3×10^{-5} ppm (3.2×10^{-4} mg/m^3) for class 3.

Overall, this analysis indicates that the lower thresholds derived for inhalation compared to the oral route are due to the respiratory tract being a sensitive target organ in inhalation studies. The consequences for route-to-route extrapolation will be explored in a future publication.

3.3. Exclusion of organophosphates or genotoxic substances

Organophosphates (OPs) as well as compounds with structural alerts for genotoxicity were excluded from the data set to evaluate whether the general inhalation TTC values for Cramer classes 1–3 are sufficiently conservative or will be shifted to higher values (Table 2). Fourteen OPs belonging to Cramer class 3 were identified and 67 genotoxic compounds: 5 in class 1, 5 in class 2, and 57 in class 3. Class 2 was not included in the analysis, as it contained less than eight data points in each data set.

The absence of OPs or genotoxic compounds does not affect the TTC for Cramer class 3, although a considerable amount of data is thereby excluded, with 40% of all chemicals in Cramer class 3 being classified as genotoxic and 7% belonging to organophosphates. In both cases, the TTC remains at 2.4×10^{-5} ppm (1.8×10^{-4} mg/m^3, respectively, Table 2), which is very close to the value of 2.2×10^{-5} ppm derived for the entire data set (Table 1). Thus, a general threshold of 2.4×10^{-5} ppm is sufficiently conservative for all chemicals in our data set.

Organophosphates do not belong to Cramer class 1; their exclusion thus does not have an influence on its threshold. Exclusion of the five genotoxic compounds, however, increases the corresponding threshold from 1.5×10^{-3} ppm (3.6×10^{-3} mg/m^3, Table 1) to 3.6×10^{-3} ppm (8.9×10^{-3} mg/m^3, Table 2).

Following the decision tree of Kroes et al. (2004), the general inhalation TTCs for non-genotoxic compounds would be $3.6 \times$

Table 2
Refinement of inhalation TTCs: exclusion of organophosphates (OPs) or substances with structural alerts for genotoxicity.

Type (N)	Cramer class	N	NOEC (ppm)					TTC		
			GM	GSD	Median	5th	95th	ppm[(*)]	mg/m^3[(*)]	μg/person/d
(–) OP ($N = 189$)	1	58	11.7	13.6	23.6	2.1×10^{-1}	338	1.5×10^{-3}	3.6×10^{-3}	71
	3	124	57.9	2.6	1.7	3.3×10^{-3}	4948	2.4×10^{-5}	1.8×10^{-4}	4
(–) Genotox ($N = 136$)	1	53	15.5	9.9	24.9	5.0×10^{-1}	338	3.6×10^{-3}	8.9×10^{-3}	180
	3	81	2.4	92.7	1.7	3.3×10^{-3}	11,111	2.4×10^{-5}	1.8×10^{-4}	4

GM, geometric mean; GSD, geometric standard deviation; NOEC, no observed effect concentration; TTC, threshold of toxicological concern.
[*] *Exposure:* 24 h/d, 7 d/week.

Table 3
Overview on current TTCs derived for consumer.

Dataset	Route	Type	Unit	*N*	TTC for Cramer class	
					1	3
RepDose	Inhalation	General	ppm[#] mg/m^3[#] μg/person/d	136[*] (203)	3.6×10^{-3} (1.5×10^{-3}) 8.9×10^{-3} (3.6×10^{-3}) 180 (71)	2.4×10^{-5} (2.2×10^{-5}) 1.8×10^{-4} (1.8×10^{-4}) 4 (4)
Carthew et al. (2009)	Inhalation	Local Systemic	μg/person/d μg/person/d	92 92	200 980	67 170
Munro et al. (1996)	Oral	General	μg/person/d	611[**]	1800	90

[#] *Exposure:* 24 h/d and 7d/week.
[*] After exclusion of substances with structural alerts for genotoxicity.
[**] Without adjustment for exposure duration of consumers (6/24 h, 7/5 d); Factor 3 applied to extrapolate from subacute and subchronic to chronic, without exclusion of substances with structural alerts for genotoxicity.

10^{-3} ppm for Cramer class 1 and 2.4×10^{-5} ppm for Cramer class 3, corresponding to 180 and 4 μg/person/d, respectively, for a 24 h exposure (Table 2).

The TTC for non-genotoxic compounds is rather low at 4 μg/person/d. The six compounds in this data set below the 5th percentile of 2.4×10^{-5} ppm are: CAS 333415 Diazinon; CAS 68359375 Cyfluthrin; CAS 57749 Chlordane; CAS 583391 mercaptobenzimidazole; CAS 77474 hexachloropentadiene, and CAS 2698411 *o*-chlorobenzalmalonitrile. All six compounds have complex structures containing multiple functional groups. Diazinon, an organophosphate, Cyfluthrin and Chlordane are used as pesticides.

4. Discussion and Conclusion

The overall objective of this report was to analyze threshold values for inhalation exposure based on the TTC concept (Munro et al., 1996; Cramer et al., 1978; Kroes et al., 2000, 2004). Table 3 gives an overview of inhalation TTCs derived in the present report. The relevance of the inhalation threshold values with regard to oral TTCs and inhalation TTCs recently derived by Carthew et al. (2009) will be discussed in the following.

The 5th percentile NOECs of classes 1 and 3 are clearly distinguished, deriving TTC values differing by a factor of at least 10. Cramer classes 1–3 overlap to some extent with respect to the NOECs for inhalation toxicity, indicating that the Cramer decision tree (Cramer et al., 1978) does distinguish broad structural classes, but fails to discriminate clearly toxic from moderately toxic and low-toxicity chemicals. The distribution of NOEC values in Cramer classes 1 and 3 shows a wide spread of up to six orders of magnitude, indicating that many moderately toxic and also some low-toxicity chemicals belong to class 3, and many toxic chemicals to class 1 (Figs. 1 and 2). In class 3, exclusion of the relatively high NOEC values of moderately toxic and low-toxicity compounds would decrease the 5th percentile and thus the corresponding TTC value. In class 1, the 5th percentile is currently triggered by the low NOEC values of toxic compounds, resulting in a very conservative threshold. Exclusion of "toxic" compounds in class 1 would increase the threshold value. By reducing the overlap a better separation of TTC values for toxic and low-toxic compounds can be achieved. This stresses the need for a better distinction of the structural classes defining toxic, moderately toxic, and low-toxicity chemicals.

In 2004, Kroes et al. published a TTC decision tree for oral exposure which guides through a number of questions to assign the appropriate TTC value to each substance. One of the first steps in the Kroes decision tree assigns a TTC of 0.15 μg/person/d to substances with a genotoxic potential based on structural alerts. For substances without such alerts, if the exposure is below a threshold of 1.5 μg/person/d, a safety concern is not expected, and there is consequently no need to classify the substance in one of the Cramer classes. Higher TTC values, to be applied to non-genotoxic substances, are based on the Cramer classification. However, the current oral thresholds for Cramer classes 1 and 3, which are 1800 μg/person/d and 90 μg/person/d, respectively, were derived without distinction of genotoxic and non-genotoxic substances (Munro et al.,1996).

Structural alerts for genotoxicity indicate that the respective compound contains reactive functional groups, especially nucleophilic or electrophilic elements, which are likely to induce genotoxicity *in vivo*. However, chemicals very often contain more than one functional group or are metabolized *in vivo*. It might therefore be that other structural parameters modify the risk for genotoxicity, e.g. by inducing rapid elimination from the organism. Therefore, an approach which excludes all compounds with structural alerts indicating a genotoxic potential may be overconservative.

The present analysis has shown that the exclusion of compounds with structural alerts for genotoxicity from class 3 did not increase the TTC (Table 2), indicating that a threshold of 2.4×10^{-5} ppm is sufficiently conservative for all class 3 substances in our data set. The six non-genotoxic compounds below the 5th percentile in class 3 have complex structural features. Three of them are used as pesticides, indicating that the TTC for Cramer class 3 would further increase if category-specific TTC values could be derived for classes of pesticides with inherent structural features or modes of action, similar to the approach proposed for organophosphates.

The exclusion of genotoxic compounds from class 1, however, increased the TTC, indicating that reactive compounds like those with structural alerts for genotoxicity should be excluded from class 1. Based on these results, we propose a general TTC of 3.6×10^{-3} ppm for Cramer class 1, excluding genotoxic compounds (Table 3), and a general threshold of 2.4×10^{-5} ppm for class 3. A threshold for Cramer class 2 is not proposed, as the Cramer decision tree groups only few substances into this class. Besides the TTCs in ppm, which allow comparison of different chemicals on a molar basis, the TTCs have been calculated also in mg/m^3. The corresponding values for Cramer classes 1 and 3 are 8.9×10^{-3} and 1.8×10^{-4} mg/m^3, which is equivalent to 180 and 4 μg/person/d, respectively. Slightly higher inhalation thresholds can be derived for the occupational situation by adjusting the exposure to 8 h/d and 5 d/week.

Overall, similar to the oral TTCs the inhalation thresholds derived are rather low, due to application of precautionary principles. Therefore, their use may be limited to conditions of low exposures, e.g. concentrations in indoor air, or emissions of compounds with low vapor pressure. Nevertheless, they provide a useful tool for assessing risks from inhalation exposure, or for prioritization, e.g. in decisions on the need for further testing.

There are several possible reasons why the inhalation TTCs derived here (Table 3) are likely to be lower than the oral TTCs derived by Munro et al. (1996). The properties of the chemicals in the inhalation data sets analyzed here may differ significantly from the chemicals analyzed by Munro et al. for oral exposure. Comparison of TTCs derived by Munro with the oral data in RepDose, however, has shown that nearly the same TTCs are obtained for both databases, although there were only few chemicals in common between both data sets (Escher et al., 2008). Therefore it is not likely, that major differences in toxicity occur if exposure is by inhalation, simply due to the differences in the chemicals analyzed. It is more likely that differences in absorption and/or metabolism (e.g. lack of first pass effect) play a role. In our analysis, absorption has been assumed to be 100% for both routes. However, absorption via inhalation may be higher than for the oral route and thus may lead to higher toxicity. For this reason, ECHA proposes a safety factor for oral-to-respiratory route extrapolation of 2 (ECHA, 2008) and only allows it for non-irritating substances. Still, our comparison shows larger differences.

Therefore, we evaluated whether the respiratory system is more sensitive to local toxic effects than the digestive tract, in which case local toxicity would trigger low threshold values. The present analysis does not intend to derive local and systemic TTCs for classes 1–3, because only active substances were analyzed. It has been shown that in inhalation studies local target organs more frequently determine the study LOEL than systemic target organs (Fig. 3). For Cramer class 1 it has been shown that indeed the NOECs and thus the corresponding 5th percentiles and TTCs based on local effects in the respiratory tract are lower than those derived from systemic toxicity. The differences between local and systemic TTCs, however, are not sufficient to completely explain the lower TTCs for inhalation. Differences in absorption as well as differences in distribution or metabolism may also account for the higher sensitivity found for inhalation.

Still, it can be concluded that for the refinement of groupings e.g. for Cramer classes 1–3 structural features inducing local effects also need to be taken into account to derive more specific inhalation thresholds. The analysis of frequently occurring structural elements of compounds presenting a large difference between local and systemic toxicity indicates that e.g. carboxylic esters could be considered. It is likely that these compounds are metabolized to carboxylic acids, which cause local irritation e.g. in the nose. Further structural elements such as α,β unsaturated carbonyls, aliphatic ethers, and secondary amines were identified. As the frequency of these structural elements is rather low, however, their potential impact has to be substantiated by analyzing a larger number of compounds.

So far, only few publications on inhalation TTCs are available. Recently, Carthew et al. (2009) derived TTCs for inhalation exposure based on a data set of 92 compounds (Table 3). The local and systemic TTC values derived by Carthew cannot be directly compared to those of the present paper, as local and systemic NOEC values were distinguished for all compounds, locally active and inactive. For dipropyl glycol methyl ether (CAS 34590948), for example, Carthew derived a local NOEC value of 430 mg/m^3, which corresponds to the highest dose tested in the cited study, as local target organs were not affected. In our analysis, dipropyl glycol methyl ether was not included in the subset of locally active substances. Furthermore, it is likely that the chemical domain of both data sets is different. Our data set covers a broad range of existing chemicals and also some pesticides, whereas Carthew's data set focused on chemicals contained in consumer products. Only 36 out of the 203 chemicals of our analysis are in common with Carthew's data set. Despite these differences, Carthew's result is consistent with our finding that local toxicity already occurs at lower NOEC values than systemic toxicity (Table 3).

Carthew described systemic TTCs of 980 and 170 μg/person/d, which were calculated similarly to the approach used by our group and Munro et al. (1996). Carthew's local TTCs, however, were normalized to a rat lung weight of 1.4 g, which cannot be directly compared to values normalized to a standard human body weight of 60 kg. Performing the same calculation as in our approach using the 5th percentile NOECs of 1.4 and 0.47 mg/m^3, thresholds of 200 and 67 μg/person/d result for classes 1 and 3, respectively (Table 3). Similar to the TTC values obtained in our analysis, the local thresholds in classes 1 (200 μg/person/d) and 3 (67 μg/person/d) are both considerably lower than those for systemic toxicity (980 and 179 μg/person/d). Thus, Carthew's analysis supports our finding that the sensitivity of the respiratory tract leads to lower thresholds for inhalation exposure, which is one reason why inhalation TTCs are lower compared to oral TTCs.

Overall, the results presented here are a first step for regulation of inhalation exposures of substances with unknown toxicity. For single compounds with known molecular weight and high vapor pressure, we propose to use the general TTCs derived in ppm as thresholds. The general TTCs in mg/m^3 may be used for aerosols and for mixtures, provided the structures of the (major) components are known. Finally, the TTCs in μg/person/d may be applicable in cases where consumers encounter exposures of short duration (less then 8 h/d) to aerosols, e.g. sprays, as already proposed by Carthew et al. (2009). However, further refinement concerning the size of the database and the definition of structural classes are desirable.

Acknowledgments

Some studies within RepDose were provided from the Toxbase database which has been developed by TNO (The Netherlands Organisation for Applied Scientific Research). Funding from Cefic LRI and the FP6 EU project OSIRIS is acknowledged.

Appendix A

CAS	Name	Author/review	Publication	Year	Study duration	Geno-toxic	OP	NOEC (ppm)		
								General	Systemic	Local
Cramer class 1										
50000	Formaldehyde	Woutersen et al.	J. Appl. Toxicol.	1989	3			1.02	1.02	1.02
57556	Propylene glycol	La Kind et al.	CRC Crit. Rev. Toxicol.	1999	2			25.7	160.7	25.7
64186	Formic acid	NTP	TOX 19	1992	2			1.33	1.33	4.00
67561	Methanol	Andrews et al.	J. Toxicol. Environ. Health	1987	1			28.2	84.6	28.2
67630	2-Propanol	Burleigh-Flayer et al.	Fundam. Appl. Toxicol.	1997	3			166.8	166.8	
71363	*n*-Butanol	Korsak et al.	Int. J. Occup. Med. Environ. Health	1994	2			8.33	8.33	
74851	Ethylene	Rhudy et al.	Toxicol. Appl. Pharmacol.	1978	2			4445	4445	
74931	Methyl mercaptan	Tansy et al.	J. Toxicol. Environ. Health	1981	2			0.34	0.34	
75070	Acetaldehyde	Woutersen and Feron	Toxicology	1987	3	×		249.8	249.8	249.8
78795	Isoprene	NTP	TRS 486	1999	3			73.5	73.5	
78831	Isobutyl alcohol	MAK, 2003	CMA, NTIS/OTS 0558855	1996	2			41.7	41.7	
78842	Isobutanal	NTP	TRS 472	1999	2	×		83.3	83.3	500.0
78933	Methyl ethyl ketone	WHO EHC 143, 1993	La Belle et al.	1955	2			130.5		
79209	Methyl acetate	EU risk assessment, 2003	HMR GmbH, Report No. 99.0011	1999	1			4.16	4.16	58.2
80626	Methyl methacrylate	NTP	TRS 314	1986	3			84.7	254.0	84.7
85687	Butyl benzyl phthalate	EU Risk Assessment, 2004	Monsanto, Report No. MSL-2713	1982	2			2.00	2.00	
91178	Decalin	NTP	TRS 513	2005	3			8.33	8.33	
96333	Methyl acrylate	Klimisch and Reininghaus	Toxicologist	1984	3			5.11	136.9	5.11
98828	Cumene	Cushman et al.	J. Am. Coll. Toxicol.	1995	2			50.0	50.00	
98839	(alpha)-Methylstyrene	NTP	TRS 543	2007	3			33.5	33.5	33.5
100378	2-Diethylaminoethanol	Hinz	Toxicology	1992	2			1.67	12.5	1.67
100403	4-Vinyl-1-cyclohexene	Bevan et al.	Fundam. Appl. Toxicol.	1996	2			41.7	41.7	
100414	Ethylbenzene	NTP	TRS 466	1998	3			24.95	24.95	24.95
100425	Styrene	Cruzan et al.	J. Appl. Toxicol.	2001	3			6.67	40.0	6.67
103117	2-Ethylhexyl acrylate	EU Risk Assessment, 2004	BASF, Report No. 50/081/8502	1989	2			4.98	4.98	4.98
105588	Carbonic acid diethyl ester	BG Chem. Toxicol. Eval. 7, 1994	Bayer, Report No. 2238	1970	1			0.65	0.65	
106990	1,3-Butadiene	MAK, 1998	Hazleton Laboratories, Report No. 2653-522/2	1981	3			337.5	337.5	1012.5
107868	3-Methyl-2-butenal	BUA Report 194, 1997	BASF, unpublished report	1994	1	×		4.84	4.84	4.84
108101	Methylisobutylketone	Phillips	Fundam. Appl. Toxicol.	1987	2			25.0	25.0	
108225	Isopropenyl acetate	MAK, 2004	BG Chem., unpublished report	2002	1			2.84	8.51	2.84
108883	Toluene	Ungvary et al.	J. Hyg. Epidem.	1980	1			22.1	22.1	
109591	Ethylene glycol (mono) isopropyl ether	ECETOC Tech. Rep. 95, 2005	CIVO-TNO, Report No. V87.299/260985	1988	1			4.99	4.99	

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Appendix A (*continued*)

CAS	Name	Author/review	Publication	Year	Study duration	Geno-toxic	OP	NOEC (ppm)		
								General	Systemic	Local
109660	*n*-Pentane	EU Risk Assessment, 2002	Exxon Biomed., Project No. 157518	1997	2			3389	3389	
109864	Ethylene glycol monomethyl ether	MAK, 1992	Chemical Manufactors Association, unpublished report	1982	2			50.0	50.0	
110805	Ethylene glycol monoethyl ether	Barbee et al.	Environ. Health Perspect.	1984	2			200.0	200.0	
111308	Glutaraldehyde	NTP	TRS 490	1999	3	×		0.02	0.13	0.02
111762	Ethylene glycol (mono) *n*-butyl ether	NTP	NIH Publication No. 00-3974	2000	3			10.4	10.4	10.4
111773	Diethylene glycol methyl ether	Miller et al.	Fundam. Appl. Toxicol.	1985	2			107.9	107.9	
111900	Diethylene glycol monoethyl ether	BG Chem. Toxicol. Eval. 15, 1999	BG Chem., Report No. BGH 33/920364	1993	1			2.58		
112072	Ethylene glycol monobutyl ether acetate	Truhaut et al.	Toxicol. Appl. Pharmacol.	1979	1			22.2	22.2	
112254	Ethyleneglycol(mono)*n*-hexylether	Klonne et al.	Fundam. Appl. Toxicol.	1987	2			10.03	10.03	
112345	Diethylene glycol butyl ether	MAK, 2008/1992	BASF AG, Project No. 50I 0030/87002	1990	2			7.91		
115071	Propylene	Ciliberti et al.	Ann. N. Y. Acad. Sci.	1988	3			199.9	199.9	
115117	Isobutylene	NTP	TRS 487	1998	3			165.6	165.6	165.6
117817	di-sec-octyl Phthalate	Klimisch et al.	Hum. Exp. Toxicol.	1991	1			0.52	10.4	0.52
121448	Triethylamine	Lynch et al.	Toxicol. Ind. Health	1990	1			41.2	41.2	
121915	Isophthalic acid	OECD SIDS, 2004	IIT Research Institute, Report No. 1301	1988	1			0.01	0.25	0.01
123386	Propionaldehyde	Melnikova and Tokanova	Gig Sanit	1983	2	×		0.21	0.21	
123864	Butylacetate	David et al.	Food Chem. Toxicol.	1996	2			84.0	84.0	84.0
140885	Ethyl acrylate	Miller et al.	Drug Chem. Toxicol.	1985	3			8.33	25.0	8.33
141322	*n*-Butyl acrylate	Reininghaus et al.	Food Chem. Toxicol.	1991	3			5.09	45.8	5.09
141435	2-Aminoethanol	Weeks et al.	Am. Ind. Hyg. Assoc. J.	1960	2			1.93	1.93	
142825	*n*-Heptane	Simonsen and Lund	Pharmacol. Toxicol.	1995	1			45.3	45.3	
592416	1-Hexene	Gingell et al.	Drug Chem. Toxicol.	1999	2			0.50	0.50	
763699	Ethyl 3-ethoxypropionate	Boggs	Appl Ind Hyg	1989	2			41.8	41.8	
1569013	Propylene glycol *n*-propyl ether	ECETOC Tech. Rep. 95, 2005	Union Carbide Corporation, Report 53–54.	1990	2			50.7	50.7	
1569024	2-Propylene glycol (mono) 1-ethyl ether	ECETOC Tech. Rep. 64/95, 1995/2005	Huntingdon Research Centre, Report BPC 46/851294	1986	2			50.5	50.5	152.6
54839246	2-Propylene glycol 1-ethyl ether acetate	ECETOC Tech. Rep. 95, 2005	Huntingdon Research Centre, unpublished report BPC 56/8655	1986	1			32.6	32.6	

Cramer class 2										
78591	Isophorone	OECD SIDS, 2005	Exxon Chemical, NTIS/OTS Mf 0206267, DOC 878210935	1968	1	×		2.04	2.04	
78853	Methacrolein	BG Chem. Toxicol. Eval. 14, 1999	Huntingdon Research Centre Ltd., BGH 50/932334	1994	2	×		0.52	0.52	2.44
79107	Acrylic acid	Miller et al.	Fundam. Appl. Toxicol.	1981	2			0.85	2.55	0.85
79414	Methacrylic acid	EU Risk Assessment, 2002	Chemical Industry Institute of Toxicology, Study No. 420-1086	1984	2			3.38	50.70	3.38
107028	Acrolein	Feron et al.	Toxicology	1978	2	×		0.07	0.70	0.07
107222	Ethane-1,2-dione	MAK, 2003	Hoechst, unpublished	1995	1	×		0.03	0.70	0.03
Cramer class 3										
51036	Piperonyl butoxide	JMPR, 1995	Bio Dynamics, Report No. 91-8333	1992	2			0.18	5.60	0.18
55312	1-Epinephrine Hydrochloride	NTP	TRS 380	1990	3			0.06	0.06	0.06
55389	Fenthion	JMPR, 1995	Bayer, Report No. 8383	1979	1		×	0.015	0.015	0.015
56235	Carbon tetrachloride	Nagano et al.	In: Chiyotani et al. (Eds.), Advances in the Prevention of Occ. Resp. Diseases, Amsterdam, Elsevier	1998	3			1.70	1.70	
58899	Lindane	MAK, 1998	Centre International d'Etudes, unpublished report	1983	2			0.03	0.03	
61825	Amitrol	WHO EHC 158, 1994	No authors given		3	×		4.85	4.85	
62737	Dichlorvos	Blair et al.	Arch. Toxicol.	1976	3	×	×	0.01	0.01	
67663	Chloroform	Templin et al.	Fundam. Appl. Toxicol.	1996	2	×		0.34	1.02	0.34
71432	Benzene	Ward et al.	Am. J. Ind. Med.	1985	2			15.0	15.00	
71556	1,1,1-Trichloroethane	Quast et al.	Fundam. Appl. Toxicol.	1988	3			510.2	510.2	
74839	Methyl bromide	Reuzel et al.	Food Chem. Toxicol.	1991	3	×		1.03	1.03	1.03
74873	Chloromethane	Pavkov et al.	Toxicologist	1982	3	×		227.6	227.6	
74964	Ethyl bromide	NTP	TRS 363	1989	3	×		33.8	33.8	202.8
75003	Chloroethane	NTP	TRS 346	1989	3	×		5002	5002	
75058	Acetonitrile	NTP	TRS 447	1994	3			200.1	200.1	
75092	Methylene chloride	NTP	TRS 306	1986	3	×		338.7	338.7	
75105	Difluoromethane	ECETOC JACC 32, 1995	ICI Central Toxicol. Lab., PAFT (CTL/P/4064)	1993	2			25028	25028	
75150	Carbon disulfide	Gottfried et al.	Neurotoxicology	1985	2			24.1	24.1	
75218	Ethylene oxide	Garman et al.	Neurotoxicology	1985	3	×		9.99	9.99	
75263	2-Bromopropane	Yu X. et al.	Environ. Res. 2001. Toxicology 1999	2001	2	×		50.0	50.0	
75354	1,1-Dichloroethene	Quast et al.	Toxicol. Appl. Pharmacol.	1977	2			4.20	4.20	
75376	1,1-Difluoroethane	ECETOC JACC 45, 2004	Haskell Laboratory for Toxicology and Industrial Medicine, unpublished report	1982	3			666.3	666.3	9995
75445	Carbonyl chloride	Franch and Hatch	J. Toxicol. Environ. Health	1986	1	×		0.01	0.04	0.01
75456	Chlorodifluoromethane	WHO EHC 126, 1991	Central Toxicol. Labortory, Report No. CTL/P/548	1988	3			9897	9897	
75525	Nitromethane	NTP	TRS 461	1997	3			18.0	18.0	
75569	1,2-Propylene oxide	WHO EHC 56, 1985	NTP (NIH No. 84-2523)	1984	3	×		29.5	29.5	29.5
75638	Bromotrifluoromethane	Scholz and	Zbl. Arbeitsmed.	1964	1			11111	5473	11111

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Appendix A (*continued*)

CAS	Name	Author/review	Publication	Year	Study duration	Geno-toxic	OP	NOEC (ppm)		
								General	Systemic	Local
		Weigand								
75650	tert-Butyl alcohol	NTP	TRS 53	1997	2			22.5	22.5	
75683	1-Chloro-1,1-difluoroethane	Seckar et al.	Food Chem. Toxicol.	1986	3			82000	82000	
76131	1,1,2-Trichloro-1,2,2-trifluoroethane	Trochimowicz et al.	Fundam. Appl. Toxicol.	1988	3			1996	1996	1996
78104	Tetraethyl silicate	Pozzani et al.	Arch. Ind. Hyg.	1951	2			44.0	44.0	
78875	1,2-Dichloropropane	MAK, 1993	Dow Chemical Company, unpublished report	1988	2	×		2.56	76.8	2.56
79016	Trichloroethylene	Prendergast et al.	Toxicol. Appl. Pharmacol.	1967	2			17.5		
79049	Chloroacetyl chloride	BG Chem. Toxicol. Eval. 12, 1998	Dow Chemical Company, unpublished report	1982	1	×		0.03	0.17	0.03
79210	Peroxyacetic acid	MAK, 1993	Heinze, Wiss Z. Humboldt Univ., Berlin; Math-Nat. R.	1984	2			9.97	9.97	9.97
79221	Methyl chloroformate	MAK, 2003	BASF, Report No. 99I0199/94006	1999	2	×		0.20	2.00	0.20
79243	Nitroethane	Griffin et al.	Ecotoxicol. Environ. Saf. unpublished report	1988	3			202.6	202.6	
88120	*N*-Vinyl-2-pyrrolidinone	Klimisch et al.	Food Chem. Toxicol. 1997	1992	3			1.61	1.61	1.61
88733	1-Chloro-2-nitrobenzene	Nair et al.	Fundam. Appl. Toxicol.	1986	1	×		0.56	0.56	
91203	Naphthalene	Abdo et al. NTP	Inhal. Toxicol. 2001 TRS 500	2001	3			3.33	3.33	3.33
95512	*o*-Chloroaniline	BUA Report 133, 1994	Bayer, Report No. 20957	1992	1	×		0.42	0.42	
95681	2,4-Xylidine	BUA Report 161, 1995	Huntington Research Center, unpublished report	1990	1	×		0.34	0.34	
95807	2,4-Diaminotoluene	Kimmerle and Solmecke	TDA Berichte, Bayer	1971	1	×		0.32	0.32	
96184	1,2,3-Trichloropropane	Johannsen et al.	J. Toxicol. Environ. Health	1988	2	×		0.78	0.78	0.78
96344	Chloroacetic acid, methyl ester	MAK, 1994	Hoechst, unpublished	1988	1	×		1.65	1.65	1.65
96457	Ethylene thiourea	MAK, 1995	BG Chem. Report No. 095760	1988	1			0.44	0.44	
98000	Furfuryl alcohol	NTP	TRS 482	1999	3			0.66	0.66	0.66
98077	(α),(α),(α)-Trichlorotoluene	MAK, 1992	Levin, FYI-OTS-0981-0122	1986	1			0.10	0.10	0.10
98873	Benzal chloride	BG Chem. Toxicol. Eval. 4, 1992	Huntingdon Research Centre Ltd.	1991	1	×		0.13	0.13	
98953	Nitrobenzene	Cattley et al.	Fundam. Appl. Toxicol.	1994	3	×		0.34	0.34	0.34
99547	1,2-Dichloro-4-nitrobenzene	Belyaev and Kuznetsov	Hyg. Sanit	1969	1	×		0.20	0.20	
100005	1-Chloro-4-nitrobenzene	Nair et al.	Fundam. Appl. Toxicol.	1986	1			0.04	0.04	
100447	(α)-Chlorotoluene	BG Chemie 48, 1997	Monsanto Company, unpublished report	1983	1	×		5.83	5.83	5.83
103719	Phenyl isocyanate	BG Chem. Toxicol.	Imperial Chemical Industries, unpublished reports	1979	1	×		0.00	0.01	0.00

		Eval. 15, 1999								
105602	Caprolactam	Reinhold et al.	Toxicol. Sci.	1998	2			0.86	26.3	0.86
106467	*p*-Dichlorobenzene	MAK, 1991/2001	JISHA, unpublished	1995	3			6.76	6.76	76.3
106887	Epoxybutane	NTP	TRS 329	1988	3	×		16.6	16.6	16.6
106898	Epichlorohydrin	Laskin et al.	J. Natl. Cancer Inst.	1980	3	×		10.04	29.86	10.04
106923	Allyl glycidyl ether	NTP	TRS 376	1990	3	×		1.69	1.69	1.69
106934	1,2-Dibromoethane	NTP	TRS 210	1982	3	×		3.34	3.34	3.34
106945	1-Bromopropane	NTP-CERHR, 2003	Clin Trials, Report No. 91190	1997	2	×		16.6	16.6	
107051	Allyl chloride	Quast et al.	Dow Chemical Company, Report 20.04.1982	1982	2	×		24.8	24.8	
107062	1,2-Dichloroethane	Cheever et al.	Fundam. Appl. Toxicol.	1990	3	×		16.5	16.5	
107255	Vinylmethylether	BG Chem. Toxicol. Eval. 5, 1993	CIVO TNO, Report No. 89.158	1989	1	×		27.8	27.8	582.3
107982	2-Propylene glycol 1-methyl ether	Spencer et al.	Toxicol. Pathol.	2002	3			300.0	300.0	
108247	Acetic anhydride	MAK, 1997	Huntingdon Research Centre Ltd., report HST 411/961219	1996	2			0.50	0.50	0.50
108316	Maleic anhydride	OECD SIDS, 2004	Goldenthal et al., Report No. 401-015	1979	1			0.17	0.17	0.17
109999	Tetrahydrofuran	NTP	TRS 475	1998	3			66.7	66.7	
110010	Tetrahydrothiophene	BG Chem. Toxicol. Eval. 7, 1994	FhG, Project No. 217280/88	1990	1			169.2	169.2	
110656	Butynediol	EU Risk Assessment, 2002	BASF, Report No. 4010226/95108	1998	1			0.02	0.24	0.02
111693	1,4-Dicyanobutane	Short et al.	J. Toxicol. Environ. Health	1990	2			0.49	0.49	
114261	Propoxur	Kimmerle and Iyatomi	Jap. J. Ind. Health 18, 375-382	1976	2			1.09	1.09	
115106	Dimethyl ether	MAK, 1988	DU PONT de Nemours, Report No 198-86	1986	3			2000	2000	
115275	Chlorendic anhydride	WHO EHC 185, 1996	Velsicol Chemical Corporation, Report No. 163.531		1			0.40	1.31	0.40
115297	Endosulfan	JMPR, 1998	Hoechst, Report No. A29823	1984	1			0.01	0.01	
116143	Tetrafluoroethylene	NTP	TRS 450	1997	3			52.2	52.2	625.8
118489	*N*-Carboxyanthranilic acid anhydride	BG Chem. Toxicol. Eval. 6, 1993	Bayer, Report No. 2143	1970	1			0.29	0.29	0.29
120821	1,2,4-Trichlorobenzene	Coate et al.	Arch. Environ. Health	1977	2			4.17	4.17	
121733	*m*-Chloronitrobenzene	Chou et al.	Toxicologist	1991	2	×		0.25	0.25	
122145	Fenitrothion	Breckenridge et al.	Toxicol. Appl. Pharmacol.	1982	1	×	×	0.03	0.03	
123773	1,1'-Azobisformamide	Medinsky et al.	Fundam. Appl. Toxicol.	1990	2	×		1.76	1.76	1.76
124403	Dimethylamine	MAK, 1993	CIIT, Docket No 11957	1990	3			3.43	51.0	3.43
126998	Chloroprene	NTP	TRS 467	1998	3			4.23	4.23	4.23
127184	Tetrachloroethene	NTP	TRS 311	1986	3			34.4	34.4	34.4
280579	Triethylene diamine	BG Chem. Toxicol. Eval. 13, 1988	RCC, Report No. 082890	1993	1			0.21	0.21	0.21
298044	Disulfoton	JMPR, 1991	Mobay Corporation, unpublished report 1131	1989	2		×	0.007	0.007	0.015
306832	1,1-Dichloro-2,2,2-trifluoroethane	MAK, 1994	PAFT, Haskell Laboratory, Report No. 669-91	1991	3	×		100.0	100.0	100.0
354336	Pentafluoroethane	ECETOC JACC 24, 1994	Japan Bioassay Laboratory, unpublished results	1993	2			25006	25006	
420462	1,1,1-Trifluoroethane	Brock et al.	Fundam. Appl. Toxicol.	1996	2			19938	19938	

(*continued on next page*)

Appendix A (*continued*)

CAS	Name	Author/review	Publication	Year	Study duration	Geno-toxic	OP	NOEC (ppm)		
								General	Systemic	Local
460731	1,1,1,3,3-Pentafluoropropane	Rusch et al.	Toxicol. Sci.	1999	2			82.1	82.1	4997.6
462066	Fluorobenzene	BG Chem. Toxicol. Eval. 13, 1998	Safepharm Laboratories, Report No. 121/194	1994	1			5.23	5.23	
509148	Tetranitromethane	Bucher et al. NTP	Cancer Lett. 1991 TRS 386, 1990	1991	3			0.17	0.17	0.17
532274	2-Chloroacetophenone	NTP	TRS 379	1990	3	×		0.05	0.05	0.05
541413	Chloroformic acid ethyl ester	Sellakumar	J. Natl. Cancer Inst. (INCI)	1987	3	×		0.51	6.08	0.51
542563	Isobutyl nitrite	NTP	TRS 448	1996	3	×		12.5	12.5	12.5
584792	Allethrin	Nakanishi et al.	Botyu-Kagaku, 35, 103–112	1970	1	×		13.5	40.4	13.5
592347	*n*-Butyl chloroformate	MAK, 2003	Huntingdon Research Centre Ltd.	1990	1	×		0.30	0.30	0.30
593602	Vinyl bromide	Benya et al.	Toxicol. Appl. Pharmacol.	1982	3	×		3.35	3.35	
611198	1-Chloro-2-(chloromethyl)-benzene	OECD SIDS, 2005	Occidental Chem. Corp., EPA doc 89-900000192, NITS/OTS 0526421	1990	1	×		0.76	0.76	0.76
811972	1,1,1,2-Tetrafluoroethane	Collins et al.	Fundam. Appl. Toxicol.	1995	3			2534	2534	
822060	Heymethylene diisocyanate	MAK, 1996	Mobay Chemical, Study No. 83-241-01, Report No. 1157	1989	3	×		0.002	0.025	0.002
836306	4-Nitrodiphenylamine	BG Chem. Toxicol. Eval. 10, 1996	Monsanto, Study No. BD-83-314	1983	1	×		0.16	0.16	
868859	Dimethyl hydrogen phosphite	SIAR, 2003	Bio Dynamics, unpublished	1982	1	×	×	0.60	0.60	0.60
872504	*N*-Methyl-2-pyrrolidone	Lee et al.	Fundam. Appl. Toxicol.	1987	3			3.39	3.39	
1634044	Methyl-tertiary-butyl ether	EU Risk Assessment, 2002	Bird et al., 1997	1997	3			396.08	396.1	
1649087	Dichlor-difluorethane	WHO EHC 139, 1992	Du Pont de Nemours, Report No. 20-88	1988	2	×		83.05	83.1	249.1
1717006	1,1-Dichloro-1-fluoroethane	Millischer et al.	Food Chem. Toxicol.	1995	3			1505	1505	
2238075	Diglycidyl ether	BG Chem. Toxicol. Eval. 3, 1992	Authors not given		2	×		0.05	0.05	
2431507	2,3,4-Trichloro-1-butene	Reuzel et al.	CIVO/TNO, Report No. V81.133/267399	1981	3	×		0.05	0.15	0.05
2524030	Dimethoxy thiophosphonyl chloride	BG Chem. Toxicol. Eval. 13, 1998	IRDC, Report No. 254-030	1981	2		×	0.01	0.01	0.01
2595542	Mecarbam	JMPR, 1980	Huntingdon Research Centre, unpublished report	1972	1		×	24.7	123.7	24.7
3689245	TEDP	Kimmerle and Klimmer	Arch. Toxicol.	1974	2		×	0.07	0.07	0.07
3811732	Sodium pyrithione	MAK, 1994	Olin Corporation, unpublished Report No MD/91213-397-042	1989	2	×		0.10	0.10	
5216251	*p*-Chlorobenzotrichloride	MAK, 1994	Occidental Chem. Corp., unpublished, EPA/OTS Doc ID: 88-920001105	1984	1			0.02	0.07	0.02

5522430	1-Nitropyrene	NTP	TOX 34	1996	2	×		0.01	0.01	0.01
6055523	1,6-Hexanediamine Dihydrochloride	NTP	TOX 24	1993	2			0.10	0.10	0.03
10265926	Methamidophos	JMPR, 1990	Bayer, Report No. 16578	1988	2		×	0.10	0.10	
17804352	Benomyl	Warheit et al., 1989	Fundam. Appl. Toxicol.	1989	2			0.42	2.11	0.42
24017478	Triazophos	JMPR, 1991	RCC, unpublished report	1987	1		×	0.01	0.01	
25311711	Isofenphos	JMPR, 1981	Bayer, unpublished report	1972	1		×	0.01	0.01	
26002802	Phenothrin	JMPR, 1980	Sumitomo Chemical Co., unpublished report	1979	1			2.56	2.56	
26447405	Monomeric Methylenediphenyl diisocyanate	EU Risk Assessment, 2005	International Isocyanate Institute, Report No 11345	1999	3	×		0.01	0.20	0.01
26530201	2-Octyl-4-isothiazolin-3-one	MAK, 1997	Rohm and Haas Comp., unpublished Report No 87R 013	1989	2	×		0.003	0.037	0.003
34590948	Dipropylene glycol methyl ether	Landry et al.	Fundam. Appl. Toxicol.	1984	2			101.5	101.5	
41198087	Profenofos	JMPR, 1990	Ciba-Geigy Ltd., Report No. Siss 5119.	1977	1		×	0.25	0.25	
52918635	Deltamethrin	JMPR, 2000	Coombs et al., no further information	1978	1			0.024	0.024	
57018527	Propylene glycol tert-butyl ether	NTP	TRS 515	2004	3			25.4	25.4	25.4
60207901	Propiconazole	JMPR, 1987	Ciba-Geigy Ltd., Report No. 79/0006	1980	2			0.25	0.25	
62610779	Methacrifos	JMPR, 1980	Ciba-Geigy Ltd., unpublished report	1977	1		×	0.51	0.51	
66215278	Cyromazine	JMPR, 1990	Ciba-Geigy Ltd., Report No. 861472	1988	1	×		0.47	0.47	
67306030	Fenpropimorph	JMPR, 1994	Ciba-Geigy Ltd., Report No.108406	1981	1			0.04	0.13	0.04
70657704	Propylene glycol methyl ether acetate	ECETOC Tech. Rep. 64, 1995	BASF, unpublished report	1984	1			93.3	93.3	
134098616	Fenpyroximate	JMPR, 1995	Bio Dynamics, Report No. 90-8290;	1991	1			0.02	0.48	0.02
2698411*	*o*-Chlorobenzalmalonitrile	NTP	TRS 377	1990	3			0.003	0.003	0.003
333415*	Diazinon	MAK, 1995	Ciba-Geigy Ltd., Project No. 891205	1990	1		×	0.001	0.001	0.006
57749*	Chlordane	JMPR, 1996	Huntingdon Research Centre, unpublished report	1984	2			0.00	0.00	
583391*	2-Mercaptobenzimidazole	BG Chemie 11, 2000	Unpublished NTP report	1984	2			0.003	0.003	
68359375*	Cyfluthrin	JMPR, 1987	BAYER, Report No 12436	1984	2			0.001	0.001	
77474*	Hexachloro-cyclopentadiene	NTP	TRS 437	1994	3			0.003	0.003	0.003

Study duration: 1, subacute; 2, subchronic; 3, chronic.
OP, organophosphate.
Genotoxic, structural alerts for genotoxicity.
* Substance below the 5th percentile NOEC in the data set "non-genotoxic compounds".

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