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A new method for setting guidelines to protect human health from agricultural exposure by using chlorpyrifos as an example

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Abstract

Introduction and Objectives. Guidelines set by various agencies for the control and management of chlorpyrifos cover a wide range of values reflecting difficulties in the procedures for their development. To overcome these difficulties a new method to set guidelines would be developed. Published data derived from epidemiological investigations on human populations would be used to develop a dose-response relationship for chlorpyrifos allowing the calculation of threshold values which can be used as guidelines.

Materials and Method. Data from the scientific literature on human populations were collected to evaluate the adverse response doses for a range of health effects. The Cumulative Frequency Distribution (CFD) for the minimum levels of adverse effects measured in terms of the Lifetime Average Daily Dose ($LADD_D$) and the Absorbed Daily Dose for neurological (ADD_{DN}) and non-neurological effects were used.

Results. Linear regression equations were fitted to the CFD plots giving R^2 values of 0.93 and 0.86 indicating a normal distribution of the data. Using these CFD plots, the chronic and acute threshold values were calculated at the 5% cumulative frequency level for chlorpyrifos exposure giving values at 0.5 µg/kg/d and 3 µg/kg/d respectively.

Conclusions. Guidelines set using this technique at the values at 0.5 µg/kg/d and 3 µg/kg/d for chronic and acute exposure respectively provide an alternative to the currently used biological endpoint and safety factor method.

Key words

Chlorpyrifos, dose-response, epidemiological study, absorbed daily dose, lifetime average daily dose, guidelines

INTRODUCTION

The common organophosphate insecticide, chlorpyrifos (*O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl phosphororthioate*), has been used for agricultural and household applications since 1965 [1], and in 2007 was the most used insecticide in the U.S., with an estimated total consumption of 7–9 million pounds [2]. Chlorpyrifos is known to have high potential for adverse effects in occupational applications, especially farmers in developing countries [3]. Chlorpyrifos can be absorbed into the human body by different pathways, including oral ingestion, inhalation and dermal absorption. It may cause neurotoxicity by inhibiting of acetylcholinesterase, and at sufficient exposures may produce adverse sub-lethal effects or death.

Policy decisions to guide risk management of chlorpyrifos are rendered difficult because national and international guideline levels vary so widely. For example the guidelines for acute exposure to chlorpyrifos vary from 3 to 100 µg/kg/d [1, 4, 5, 6]. The highest level for acute exposure was set by the World Health Organization/Food and Agriculture Organization (WHO/FAO) at 100 µg/kg/d, and the lowest level by the Agency for Toxic Substances and Disease Registry (ATSDR) at 3 µg/kg/d. Similarly, the governmental or official guidelines for chronic exposure to chlorpyrifos range from 0.3 to 10 µg/kg/d. The reason for this degree of guideline variation is that the agencies have used different biological endpoints and safety factors to establish their guideline values.

The acute Reference Dose (aRfD) recommended by the US EPA (500µg/kg/d) is based on the dose that causes inhibition of rat plasma. However, use of that endpoint has been criticized as being too conservative, since it is not associated with any clinical evidence of chlorpyrifos exposure [7]. The Agency for Toxic Substances and Disease Registry (ATSDR) has selected the AChE inhibition dose with the surrogate animal brain at 30µg/kg/d to calculate the acute Minimum Risk Level (MRL) [1]. Other agencies, such as WHO and the Australian National Registration Authority for Agriculture & Veterinary Chemicals (NRA), have chosen the dose that causes observed biological effects on humans as the basis for establishing acute guideline values.

The probabilistic dose-response assessment used in ecological risk assessment has been also described by Solomon et al. (2002) [8], and this approach has been used to evaluate water quality as well as toxic effect data. For example, Solomon et al. (2002) [9] made SSD plots of the NOAEL for many different species of aquatic organism using the Lethal Concentration/Effective Concentration in water of permethrin. They applied the probabilistic technique to ecological dose-response data, in general, and specifically to evaluate the ecological risk due to chlorpyrifos in North American aquatic environments.

Human epidemiological data have been processed by using the probabilistic technique to evaluate human health risk [10, 11, 12]. With this technique the cumulative frequency of doses corresponding to adverse human biological effects

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were plotted and evaluated. There is a considerable volume of dose-response data on chlorpyrifos for human adverse responses as observed in epidemiological investigations. The use of human data to develop guidelines is preferred to using responses from surrogate animals or plasma, because it directly relates to human health.

The objective of this research was to develop a new method for the setting of guideline values which would utilise human data directly and thus not involve the use of surrogate test animals and safety factors. This new method would require the development of dose-response relationships from human data for an example chemical and for the reasons outlined above chlorpyrifos was selected for this purpose.

Principals of the probabilistic method of guideline development. The data available is principally as a result of epidemiological investigations of the adverse effects of chlorpyrifos on human populations. This data suffers from several deficiencies for use in the evaluation of dose – response relationships. There is no single investigation which is designed to meet this purpose which has a consistent and measured exposure pattern and methods to evaluate adverse health effects. The available data results from investigations having many different designs with different exposure patterns, different adverse effects reported, different populations and exposure for different time periods. Nevertheless there can be many diverse sources of data which can be collated to give support to general relationships which may be established. This is the case with chlorpyrifos which has a considerable volume of epidemiological data available which can be processed to establish general relationships. In this study, the guideline for chronic lifetime exposure was set up from a wide range of doses; on the other hand the guideline for acute exposure was set up from neurological adverse effect which is the typical acute effect of Organophosphate compound, and we suggested the usage of internal dose which is the combination of all exposure routes. The durations of exposure here were set up for two stages: lifetime exposure & acute exposure.

This data can be collated and organised into a sequence of exposures and adverse effects which are of increasing magnitude and a probabilistic plot can be made from it. Such probabilistic plots could be expected to show deviations from the normal distribution since the sources of data are diverse. However the data set could be rejected as unsuitable if the deviations were too great. On the other hand if the plots were derived from many investigations and were linear, suggesting a normal distribution, they could be used as a dose – response relationship.

In the derivation of guideline values we are concerned with the threshold levels below which adverse effects are not observed and above which there are adverse effects. Thus from the minimum levels of individual investigations a probabilistic relationship can be derived and a linear regression relationship calculated. Using the linear regression relationship a value of the threshold at the 5% cumulative probability level can be calculated. This level is usually accepted as the lowest level that can be reliably estimated from this data using the probabilistic plots. The 5% level can serve as a guideline value since no reliable adverse effects have been observed in any human population resulting from any investigations below this value and adverse effects have been observed in several investigations above this value.

MATERIALS AND METHOD

Data Collection. Dose – response data on the specific adverse health effects from chlorpyrifos recorded from human epidemiological studies on human populations where the exposure data was available. The databases searched included the following: TOXNET, MEDLINE, PUBMED, and Toxicological Databases in the Occupational/Environmental Health Directory.

The adverse effects identified from the literature search were grouped either as neurological or non-neurological effects. Non-neurological effects included effects such as developmental, reproductive, among other target-organ effects. Only epidemiological studies that illustrated an association between specific exposure doses of chlorpyrifos, or its biomarker, 3,5,6-trichloropyridinol (TCP), were included.

Estimations of Doses: ADD, and LADD. ***Absorbed Daily Dose (ADD).*** The doses obtained from epidemiological studies on human populations were converted to ADD using appropriate equations, the specific nature of which depended on the route of exposure. Where dose was reported as intake dose, it was converted to ADD by using Equation 1, in which the Absorption Factors were based on the specific route of exposure.

$$ADD\ (\mu g/kg/d) = Exposure\ Dose . Absorption\ Factor$$

Equation 1

where, ADD is Absorbed Daily Dose; Exposure Dose, intake dose; the Absorption Factor for chlorpyrifos was assumed to be 70% for oral exposure [13], 1% for dermal exposure [14], and as assumed 100% for inhalation exposure.

If the source of a dose was reported to be TCP, a major metabolite of chlorpyrifos, the estimation of chlorpyrifos Absorbed Daily Dose (ADD) used the approach described by Mage et al. [15] and Curwin et al. [16]. Eaton et al. (2008) [17] point out that with some populations, particularly nonoccupationally exposed populations, there may be errors due to the possible occurrence of TCP in the urine due to its background presence in food and other sources. However this comment is not applicable to exposure where TCP has been measured both before and after an event or in a background sample. The results used in this evaluation were from studies where the TCP levels in urine were corrected for the presence of TCP in the background. The ADD (μg/kg/day) was calculated from a combination of individual urinary TCP concentration and the individual daily creatinine excretion rate (g/d) calculated from their age, gender, height and weight of the exposed individual, on a body weight basis.

$$ADD\ (\mu(g/kg)/d) = (C.C\_n.CF.R\_mw)/BW$$

Equation 2

where, ADD is Absorbed Daily Dose (μg/kg/d); C, concentration of TCP in urine per gram creatinine (μg/g creatinine); Cn, calculated mass of creatinine excreted per day (g/day); CF, correction factor of chlorpyrifos 1.4 (approximately 70% of chlorpyrifos is excreted as TCP in urine); R_{mw}, the ratio of parent chlorpyrifos and TCP metabolite molecular weights; BW, body weight (kg).

Lifetime Average Daily Dose (LADD). The Lifetime Average Daily Dose (LADD) that related to adverse effects from epidemiological studies was estimated from ADD by using the following equation.

$$LADD\ (\mu g/kg/d) = (ADD.EF.ED)/\ AT$$

Equation 3

where, ADD (μg/kg/d) is the Absorbed Daily Dose of chlorpyrifos for the pesticide applicator; EF, the exposure frequency (spray events or contact events/year); ED, the exposure duration (42 working-years: for 18–60 year-olds; or 70 years for a lifetime); and AT, the average time (70 years × 365 days/year).

Probabilistic dose-response assessment of chlorpyrifos. A cumulative frequency distribution (CFD) was obtained by plotting Cumulative Frequency (CF) against the ADD, and LADD using Microsoft Excel 2007, in which the CF was calculated as cumulative percents of individual doses. This allows the frequency of chlorpyrifos doses at different levels to be evaluated by fitting a regression line [18]. The different levels of probability correspond to the different levels of observed adverse biological effects. The 5th, 50th and 95th percentile values of ADD, and LADD were used to examine the dose at the low, median and high exposure level. It has been demonstrated that there are not usually sufficient data points obtained from epidemiological studies to reject linearity, and the empirical evidence for nonlinearity may be very weak. In addition, there is often no good biological reason for rejecting linearity. Therefore, the linear regression was preferable for this study. The linear regression between CF and doses (ADD, and LADD) was analysed, in which the assumptions of the linear regression model such as normality and linearity were checked from the residual analyses using Normal P-P Plot and scatterplot for regression standardized residual.

Dose-response relationship for chlorpyrifos from human epidemiological investigations. Neurological Effects. The doses observed to produce human neurological effects are shown in Table 3. Coulston et al. [19] and Nolan et al. [13] reported the threshold dose for males for inhibition of plasma BuChE. Also Kisicki et al. conducted study with healthy volunteers [6] in which a single mg/kg dose of chlorpyrifos represented the threshold for erythrocyte AChE inhibition.

Steenland et al. [20] conducted a retrospective cohort study of the association between chlorpyrifos exposure and neurological function effects with termiticide applicators. Adverse symptoms such as memory problems, emotional problems, fatigue, loss of muscle strength were reported as well as significant declines in nerve conduction velocity, arm/hand tremor, vibrotacticle sensitivity, vision, smell, visual/motor skills, and neuro behavioural skills [16]. Dick et al. [21] found acute sensory-motor effects at an average urinary TCP level of 200 μg/g creatinine.

Several studies on the relationship between chlorpyrifos exposure and neurological effects were conducted with chemical industry workers. Albers et al. performed a prospective cohort study over a period of two years to evaluate any association between chlorpyrifos exposure and clinically evident central nervous system dysfunction for manufacturing versus control workers [22, 23]. In another study, Albers et al. [23] found a dose-effect relationships for peripheral nerve electrophysiologic effects in chemical workers occupationally exposed to chlorpyrifos. Garabrant et al. (2008) found BuChE inhibition decreased by 21% for each unit above the urinary TCP level of 110 μg/g creatinine [24]. Farahat et al. [25] recently determined that the average urinary TCP concentration inflection point for BuChE inhibition was 114 μg/g creatinine.

Non-neurological organ system toxicity. The results of investigations of non-neurological organ tocicity are summarised in Table 4. Reproductive effects have been evaluated by Meeker et al. [26] who reported a relationship between urinary metabolites of chlorpyrifos, and both the quality of human semen quality and DNA damage in sperm. Borderline significant associations existed for decreased sperm concentration and motility with the highest urinary TCPy of 35.1μg/g creatine.

Meeker et al. [27] also investigated the reproductive effects associated with chlorpyrifos exposure by evaluating the relationship between urinary TCP levels and male reproductive and thyroid hormones. The highest TCP quintile was associated with a testosterone decline of 83 ng/dL [28]. Also [20] found that the maximum urinary TCP level was 35.1 μg/g creatine (Tab. 4).

Berkowitz et al. [29] conducted a prospective cohort study to evaluate the relationship between *in-utero* pesticide exposure, and two key effect parameters (viz., maternal paraoxonase activity, and head circumference) of mothers and infants. Urinary TCP concentrations over the limit of detection (LOD) level (11.0 μg/L) was significantly associated with reduced head size of infants delivered by women who had low maternal paraozonease polymorphisms (PON1). The creatinine-adjusted urinary TCP level estimated from the individuals showing residues that exceeded the LOD value (11.0 μg/L), was 7.9 μg/g creatinine [21].

Dose-response assessment of chlorpyrifos. Cumulative frequency of dose occurrence. The specific adverse health effects derived from human epidemiological studies are summarized in Table 1 and 2 and in Figure 1. The doses associated with different types of adverse effects in terms of $LADD_D$, and ADD_{DN} were taken as a single value if they were reported as single values; otherwise, the minimum doses were used if data were reported as a range of values. These doses were categorized according to broad adverse effects such as neurological, reproductive, developmental effects and are summarized in Figure 1.

The data summarized in Figure 1 is plotted in probabilistic terms as cumulative frequency of exposure doses to chlorpyrifos that corresponds to the adverse health effects presented in Figure 2. The residual analyses ensured no violation of the asumptions of normality and linearity for both doses ($LADD_D$ and ADD_{DN}). Linear regression relationships clearly existed, with the high correlation of 0.82. The equation was as follows:

$$CF\ (\%) = 55.6\ Log(LADD_D) + 22.8 \qquad (r^2,\ 0.86)$$

Equation 4

The $LADD_{D95}$ was 19.9 μg/kg/d, the $LADD_{D50}$ 3.2 μg/kg/d and the $LADD_{D5}$ 0.5 μg/kg/d. The neurological effects observed are a surrogate for the acute adverse health effect

Table 1. Dose-response of chlorpyrifos for neurotoxicity from epidemiological studies with human populations

References	Research subjects (mean age)	Single dose (µg/kg)	Ingestion dose (µg/kg/d)	TCP in urine (µg/L, or µg/g creatinine)	Absorbed Daily Dose estimated from TCP (ADD_D)* (µg/kg/d)	Lifetime Average Daily Dose ($LADD_D$)** (µg/kg/d)	Biological effects
Steenlan et al., 2000 [20]	Termiticide applicators			629.5 µg/L	35.7	11	memory problems, emotional states, fatigue, and loss of muscle strength
Dick et al., 2001 [21]	Pesticide applicators			200 µg/g	15.6	4.9	Sensory and motor effects
Albers et al., 2004a [22]	Chemical workers			192.2 µg/g	15.6	6.2	BuChE inhibition
Garabranti et al., 2008 [24]	Chemical workers			>110 µg/g	5	2	Bu ChE inhibition RBC ChE inhibition
Albers et al., 2007 [30]	Chemical workers			576–627 µg/day	15.6–17	6.2–6.7	Electrophysiology suggestive of subclinical neuropathy
Farahat et al., 2011 [25]	Agricultural workers			3,161 µg/g	181	3	AChE inhabitation

*Estimated using Equation 2
**Estimated using Equation 3

Table 2. Dose-response of chlorpyrifos for non-neurotoxicity from epidemiological studies with human populations

References	Research subjects	TCP in urine µg/g creatinine	Absorbed Daily Dose estimated from TCP (ADD_D) (µg/kg/d)	Lifetime Average Daily Dose ($LADD_D$) (µg/kg/d)	Biological effects
Meeker et al., 2004 [26]	Adult males	35.1	2.6	1.6	*Reproductive effect*: human sperm quality, DNA damage in sperm
Meeker et al., 2006 [27]	Adult males	35.1	2.6	1.6	*Endocrine effect*: Decrease Testosterone; Increase TSH, and decrease thyroid hormone free T_4.
Berkowitz et al., 2004 [29]	Pregnant women	>11.0 µg/L	0.5	0.3	*Developmental effect*: Decrease head circumference among infants

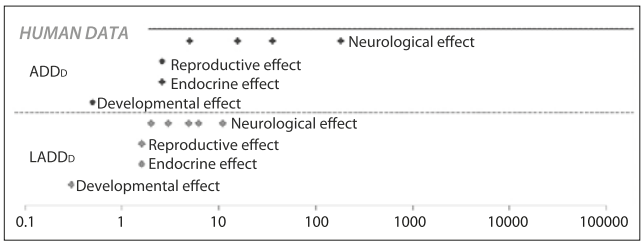

Figure 1. Doses of Chlorpyrifos which produce adverse biological effects, derived from human epidemiological data (ADD_D and $LADD_D$ from Tables 1, 2)

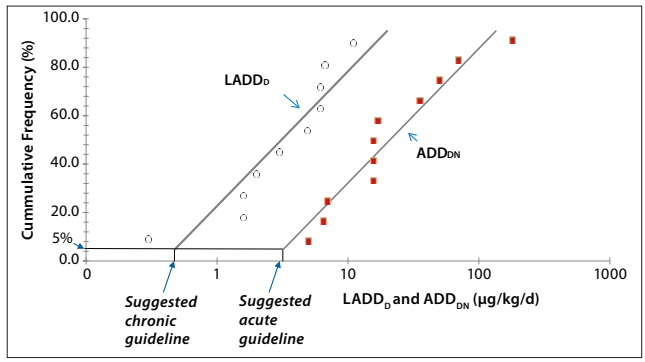

Figure 2. CFD of human adverse effect doses, derived from Figure 1 using data on ADD_{DN} and $LADD_D$ from Tables 1, 2

of chlorpyrifos due to the ability of the chlorpyrifos-oxon metabolite to inhibit acetylcholineseterase. The Absorbed Daily Dose corresponding to neurological effects (ADD_{DN}) is summarized in Figure 1 from the data in Table 1. The cumulative frequency distribution (CFD) of the Absorbed Daily Dose corresponding to neurological effects (ADD_{DN}), is presented in Figure 2, the regression equation for which is shown below:

$$CF(\%) = 55.5Log(ADD_{DN}) - 23.5 \qquad (r^2, 0.93)$$

Equation 5

The ADD_{DN95} (126 µg/kg/d) was about equal to ADD_D, while the 50th percentile of (20 µg/kg/d) and 5th percentile (3.2 µg/kg/d) of this dose were nearly 2 and 3 times higher than the general ADD_D.

Relationship of guideline values and human epidemiological study results. The chronic guideline values developed by various agencies were compared using the Lifetime Average Daily Dose ($LADD_D$) (Fig. 3), and the acute guideline values were compared with Absorbed Daily Dose (ADD_{DN}) number for observed acute human neurological effects (Fig. 4). The highest chronic effect guideline was 10 µg/kg/d as recommended by WHO/FAO. This value falls at the 80th percentile of the $LADD_D$. The chronic guidelines recommended by the US EPA (i.e.,0.3 µg/kg/d) was less than the minimum $LADD_D$ value observed from the epidemiological studies. However, the guideline value recommended by ATSDR was 1 µg/kg/d, and fell at the 23th percentile of the $LADD_D$. The guideline value for sensitive groups of the populations was 0.03 µg/kg/d, and fell well below the minimum value for any dose observed to have an adverse effect on a human population (Fig. 3).

The highest value of any acute guideline value was 100 µg/kg/d, as recommended by WHO/FAO. This value fell at the 88th percentile of ADD_{DN}. The acute guideline values recommended by US EPA and ATSDR were 5 and 3 µg/kg/d

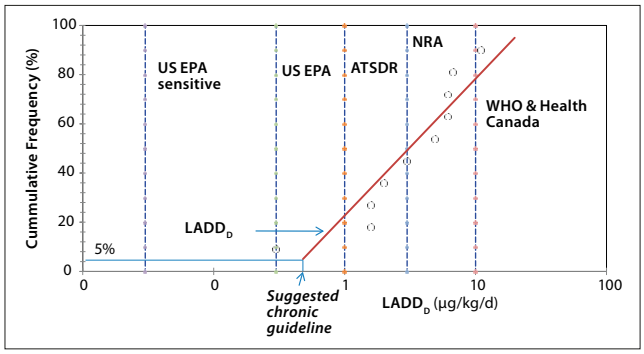

Figure 3. Lifetime Average Daily Dose (LADD$_D$) values, derived from epidemiological data compared with chronic guideline values from various agencies

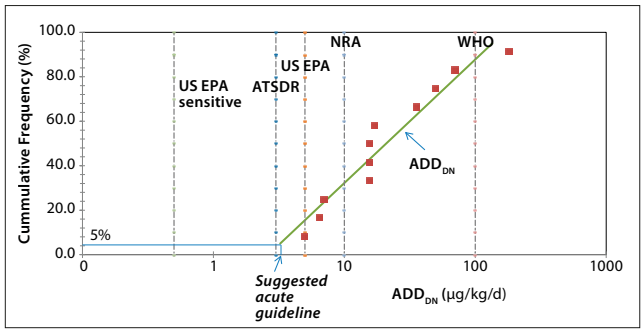

Figure 4. Comparison of Absorbed Daily Dose for neurotoxicity (ADD$_{DN}$), derived from epidemiological data with acute guidelines from various agencies

respectively. These fell at the 15th and the 3th percentile of ADD$_{DN}$, respectively. The acute guideline value recommended by the US EPA for sensitive populations was 0.51 µg/kg/d, and is 6 times less than the 5th percentile of ADD$_{DN}$ (Fig. 4).

Suggested guidelines based on the threshold doses. The threshold value derived at the 5th percentile of the LADD$_D$ can be considered as a guideline value since it represents the lowest level of exposure that is associated with any observed adverse human health effects. This value (0.5 µg/kg/d; 95%CI,-2.13–2.63) was calculated from Equation 5 (Fig. 2) and is similar, but slightly higher than the chronic Reference Dose (cRfD) recommended by the US EPA, at 0.3 µg/kg/day. Similarly, an acute guideline value can be derived at the 5th percentile of ADD$_{DN}$ at 3 µg/kg/d (95%CI, 2.90–3.09) using Equation 8. This is a surrogate for acute neurological effects observed from human epidemiological studies (Fig. 5). This suggested guideline value is the same as the acute Minimum Risk Level (MRL) that is recommended by ATSDR.

To address sensitive populations, the US EPA has applied an additional safety factor of 10 to their guideline value. This translates to a chronic guideline value of 0.05 µg/kg/d and an acute guideline value of 0.3 µg/kg/d for sensitive populations (e.g., infants and women of reproductive ages at 13–50 years. The values suggested for sensitive populations compare well to the doses at which reproductive and developmental effects were observed in human epidemiological studies (Fig. 1). However, additional epidemiological data are needed to establish guidelines that are based on actual human data.

CONCLUSIONS

The main toxic effect of chlorpyrifos is acetylcholinesterase inhibition, which results in neurological effects, and adverse reproductive and developmental effects. The data on dose-response effects was plotted as CFDs with the following linear regression equations for adverse human health effects:

$$CF\ (\%) = 55.6 \log(LADD_D) + 22.8 \qquad (r^2, 0.86)$$

For chronic effects over a lifetime

$$CF(\%) = 55.5 Log(ADD_{DN}) - 23.5 \qquad (r^2, 0.93)$$

For acute effects

These equations were used to calculate the threshold dose values for chlorpyrifos exposure at the 5% cumulative level. The 5% levels can be used as guidelines thus the LADD$_D$ gave a chronic guideline value of 0.5 µg/kg/d and the ADD$_{DN}$ gave an acute guideline value of 3 µg/kg/d.

Although it has been aware of the advantages of traditional regulatory toxicological approach, compared to the method using epidemiological evidence such as higher validity, accuracy and repeatability of toxicological data due to controlled experiment and administration and durations of exposure routes, the different guideline values recommended by various agencies have resulted from the use of different biological endpoints and safety factors. The new method proposed in this paper utilises human data based on epidemiological investigations obviating the use of biological end points and safety factors. However, further information on human is needed to confirm the appropriateness of these values and to set more firm guideline values for sensitive population groups. In addition, the potential errors that might be occured by using epidemiology data should be examined carefully for similar evaluation, including the uncontrolled/unmeasured confounders, limited information on routes/duration of exposures, especially for certain study designs (e.g. cross-sectional study) and statistical limitations (e.g. assumptions on statistical models and random errors), and non-differential measurement errors.

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