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ABSTRACT

Regulatory information requirements for pesticides call for submission of acute systemic toxicity data for up to three different exposure routes (oral, dermal, inhalation) for both active ingredients and formulated products. Similar multi-route testing is required in the European Union and elsewhere for industrial chemicals. To determine the value of acute toxicity testing by more than one route, oral-dermal and oral-inhalation concordances among regulatory classifications were examined for large data sets of chemicals and pesticide active ingredients. Across all sectors examined, oral acute toxicity classifications for pure active substances were more severe than those derived from dermal data in more than 98% of cases, which calls into question the value of routine dermal route testing for acute toxicity. Oral classifications were equivalent to or more severe than for the inhalation route for 83% of industrial chemicals and for 48% of pesticides examined.

1 Introduction

Acute toxicity refers to adverse effects occurring following a single exposure to a substance or following multiple exposures within 24 hours. In the area of regulatory toxicology, acute toxicity studies are the longest standing class of toxicity test, dating back to the “lethal dose 50 percent” method developed by Trevan (1927). However, the use of lethality as an endpoint has long been a subject of controversy on both ethical/animal welfare and scientific grounds (Balls, 1991; Robinson et al., 2008; Seidle et al., 2010). Pharmaceutical companies have stated that “these studies have limited value in terms of pre-clinical and human safety assessment compared to the substantial adverse effects experienced by some of the animals” (Robinson et al., 2008), and this sector itself has recently moved to discontinue the routine requirement for stand-alone acute toxicity studies (ICH, 2009). Systemic acute toxicity studies

nonetheless remain a common feature in a number of regulatory frameworks and voluntary initiatives, such as high production volume (HPV) chemicals programs. Available statistics indicate that more animals have been used in recent years in assessment of this endpoint than in any other single area of toxicology (EC, 2007).

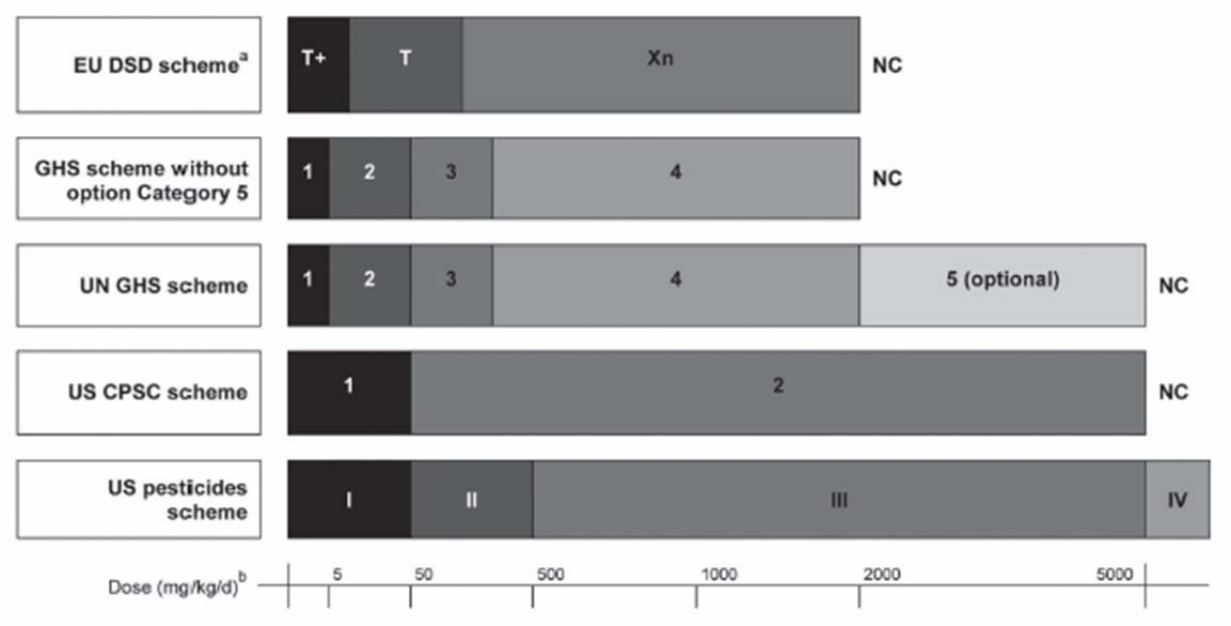
In certain sectors, regulatory information requirements prescribe testing for acute toxicity by up to three different exposure routes (oral, dermal, inhalation), and in some cases, for both individual substances/ingredients and formulated products/articles. This generally is the case for agricultural and plant protection chemicals and biocidal products (collectively referred to as “pesticides”) due to their intended biological activity and toxic mode of action, as well as the potential for human exposure via multiple routes (e.g., oral ingestion of residues on food; potential dermal and/or inhalation exposure during the application process, and in other occupational scenarios). Requirements for industrial chemicals are more variable from country to country but in certain cases may also prescribe acute systemic toxicity testing by more than one exposure route. For example, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation in the European Union (EU) requires single-route acute toxicity data for all substances manufactured or imported in volumes of more than one metric ton per annum (tpa), and data for a second route for substances in the ≥10 tpa band (OJ, 2007). A detailed review of regulatory requirements and other drivers for acute toxicity test data across industry sectors and major international markets has been published elsewhere (Seidle et al., 2010).

A key finding of the aforementioned review is that the principal use of acute toxicity data is to support regulatory classification and hazard labeling decisions (although it is recognized that these data can also be used to derive safe use threshold levels, e.g., Derived No Effect Level (DNEL) or Acute Exposure Guideline Level (AEGL)). Frameworks for classification and labeling differ somewhat among countries/regions, and sometimes among authorities within the same country. The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) was developed under the auspices of the United Nations (UN, 2007) to promote increased regulatory consistency and efficiency among countries and sectors. Figure 1 illustrates the different hazard class cut-offs for acute oral toxicity between the GHS and its most common variants in use in Australia, Canada, and the EU (OJ, 2008), as well as the former EU scheme under the Dangerous Substances Directive (OJ, 1967), and schemes used by United States agencies charged with worker protection (OSHA, 2009) and the regulation of consumer products (CPSC, 1973) and pesticides (EPA, 2004). A key difference among these schemes is the prescribed limit dose (i.e., 2,000 mg/kg or 5,000 mg/kg), beyond which a substance is not required to bear a hazard label for acute toxicity. The GHS designates testing beyond 2000 mg/kg as optional and discouraged on animal welfare grounds (UN, 2007), and the US Occupational Safety and Health Administration (OSHA, 2009) adds that exposures of this magnitude are not likely to be encountered in the occupational setting. For these reasons, countries adopting the GHS are for the most part adopting a 2,000 mg/kg limit dose for acute oral toxicity. Figures 2 and 3 compare selected classification schemes for acute dermal and inhalation toxicity.

Notwithstanding differences among national and sector-specific classification systems, it is common to use the lowest available oral or dermal lethal dose (LD50) or inhalation lethal concentration (LC50) value to assign a substance or article to a hazard category (OJ, 2008), and label warnings normally reflect the most severe hazard category (UN, 2007; OSHA, 2009). Thus, if it were possible to identify an exposure route that is consistently more or less sensitive than another and is relevant to a particular exposure scenario, multi-route animal testing would not be necessary. The “3Rs” principle of replacement, reduction, and refinement of animal use (Russell and Burch, 1959) is a longstanding tenet of sound science, and it seems especially apt in the case of toxicity studies that involve not only death as a primary endpoint in most cases but also testing of the same substance using multiple routes of exposure and/or

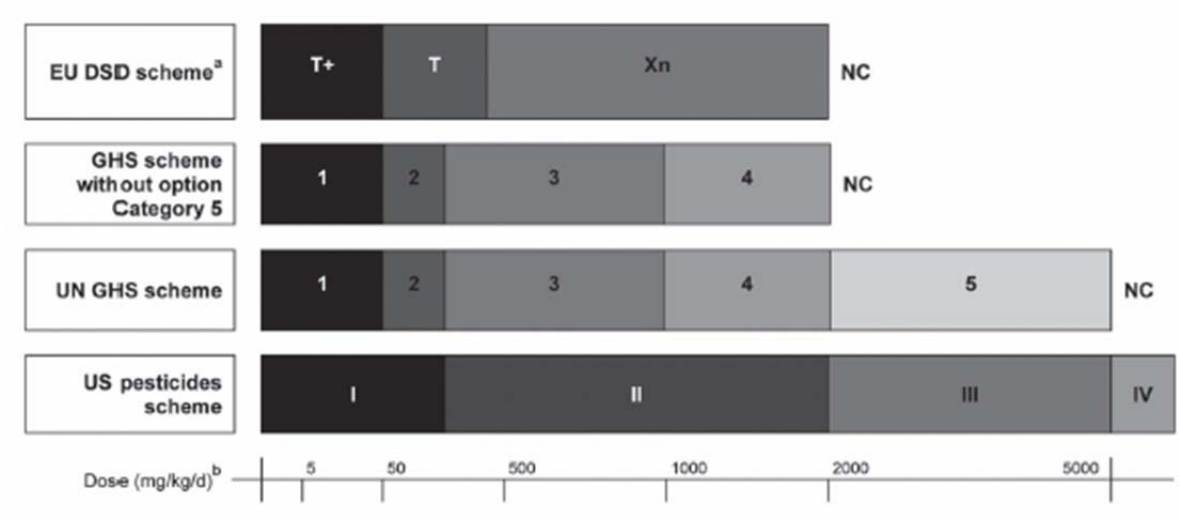
species. In some parts of the world, the minimization of animal testing is a legal requirement, as exemplified by Article 7.2 of EU Directive 86/609/EE C for the protection of animals used for experimental and other scientific purposes (OJ, 1986), and Article 13 of REACH, which specifies that hazard information shall be generated whenever possible by means other than vertebrate animal tests.

Fig. 1: Comparison of hazard classification schemes for acute oral toxicity



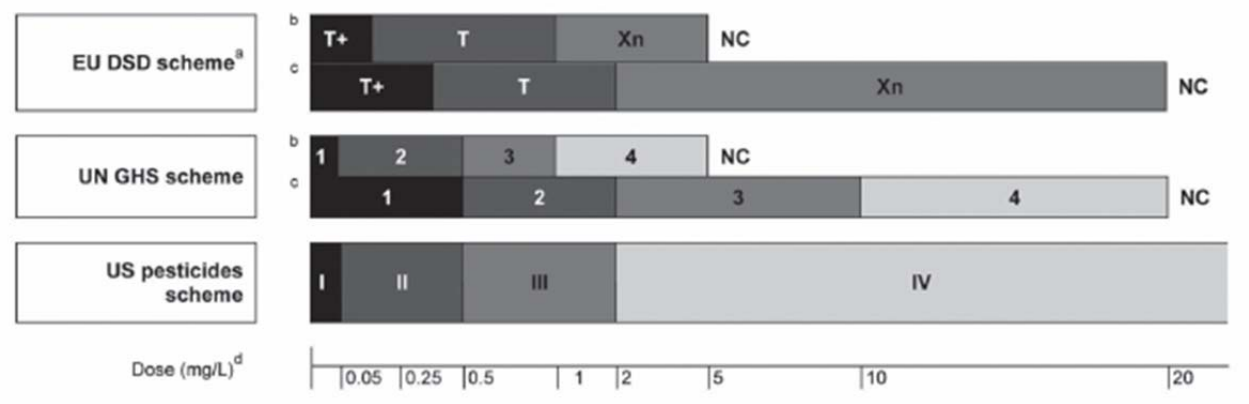
[a] NC = no classification required; Xn = harmful; T = toxic; T+ = very toxic
[b] Not drawn to scale

Fig. 2: Comparison of hazard classification schemes for acute dermal toxicity



[a] NC = no classification required; Xn = harmful; T = toxic; T+ = very toxic
[b] Not drawn to scale

Fig. 3: Comparison of hazard classification schemes for acute inhalation toxicity



[a] NC = no classification required; Xn = harmful; T = toxic; T+ = very toxic
[b] Classification bands for dusts and mists (solid and liquid aerosols)
[c] Classification bands for vapors and gases
[d] Not drawn to scale

To date, a handful of retrospective reviews of acute oral and dermal toxicity classifications have been reported in the literature. In 1998, investigators from the UK Health and Safety Executive (Indans et al., 1998) presented the results of an analysis of acute toxicity classifications for 438 industrial chemicals notified in the EU for the period 1984 to 1997. This analysis found that only four of these substances were positively classified for acute dermal toxicity, and for only one of these did the dermal study lead to a more severe classification than the oral study. In 2007, the UK Pesticide Safety Directorate (Thomas and Dewhurst, 2007) examined unpublished acute oral and dermal toxicity data for 195 pesticide active ingredients and 3,111 formulated products, concluding that the dermal study adds little if anything to the database on pesticide active substances, and that a similar result was indicated for formulations. A more recent paper by Creton and colleagues (2010) arrived at the same conclusion following the review of a slightly expanded data set of pesticide active ingredients. This paper builds on the above analyses using a significantly expanded data set of industrial chemicals, as well as pesticide active ingredients from non-EU sources that have not been considered in the aforementioned reviews. We also examine concordances among oral and inhalation route classifications, and consider the impact on our conclusions of the prevalence of positively and negatively classified substances within the database. This publication contributes to the efforts of the Acute Toxicity Task Force of the European Partnership for Alternative Approaches to Animal Testing (EPAA) to identify opportunities for application of the 3Rs in this area.

2 Methods

2.1 Chemicals dataset Data for industrial chemicals were obtained from the EU New Chemicals Database (NCB), a proprietary repository of toxicity information for all substances notified to European authorities since 1981 (i.e., from the entry into force of the sixth amendment to the former EU Dangerous Substances Directive 67/548/EEC). Substances exempted from notification include pesticides, cosmetics, pharmaceuticals, foodstuffs, radioactive materials, wastes, and substances used in scientific research. When accessed on June 11, 2008, the NCD contained 7,812 notification Methylidossiers, representing 4,946 substances notified in Europe since 1981. For the analysis performed in this study, all substances were included that possessed a classification after acute systemic exposure in rats *via* oral, dermal and/or inhalation routes (3,317 in total). Substances excluded were those for which the LD/LC50 values reported

were not consistent with the classification (e.g., an oral LD50 >800 mg/kg being designated “non-classified”). Of the 3,317 substances with acute toxicity data for at least one relevant exposure route, 1,990 (60%) also were tested by a second route, including 1,737 substances with both oral and dermal data and 81 substances with both oral and inhalation data. Following the application of exclusion criteria, a total of 1,569 substances were used for the oral-dermal concordance assessment, and 71 substances were used for determining oral-inhalation concordance.

Regulatory classifications listed in the NCB are based on the now historic EU Dangerous Substances Directive (DSD) scheme (since superseded by EU Regulation 1272/2008 on Classification, Labeling and Packaging of Substances and Mixtures (CLP; OJ, 2008). A decision against converting individual classifications from the four category DSD system to the up to 5-6 category GHS scheme (see Fig. 1-3) was made on the basis that most dermal studies (97%) were conducted as limit tests using a 2,000 mg/kg cutoff, which precludes a direct comparison against oral studies in which dosing up to or beyond 5,000 mg/kg was performed. As such, substances with oral and dermal LD50 values in excess of 2,000 mg/kg are considered for the purposes of this analysis as being “non-classified” for acute toxicity.

2.2 Pesticides dataset Data for agrochemical and biocidal active ingredients were obtained through systematic reviews of the following publicly accessible online databases and information repositories maintained by regulatory authorities and intergovernmental bodies: Working Documents of the EC Standing Committee on the Food Chain and Animal Health in view of the inclusion of plant protection active substances in Annex I of Directive 91/414/EEC (EC, 2009a; EC, 2009b) Assessment Reports concerning inclusion of biocidal active substances in Annex I or IA to Directive 98/8/EC; European Food Safety Authority (EFSA, 2009) Draft Assessment Reports for plant protection active substances; US Environmental Protection Agency (EPA, 2009; 2010) Reregistration Eligibility Decision documents and Fact Sheets on new active ingredients; and the International Program on Chemical Safety (IPCS, 2009) INCHEM database. LD/LC50 values were collected if available for at least two of the three relevant exposure routes (oral, dermal, inhalation) using mammalian species and procedures specified in current Organization for Economic Cooperation and Development (OECD, 2009) test guidelines, together with CAS registry number, broad product type (e.g., antimicrobial, insecticide), and physical state descriptor. In the case of regulatory decision documents, only a single value was normally reported for each exposure route, which may reflect a pre-selection by authorities of the (generally lowest) LD/LC50 value from among two or more possible choices. The LD/LC50 values in the summary documents were taken at face value without attempts at independent confirmation. Given the size of the database, it was considered that any overall conclusions would not be unduly confounded by an occasional error in the production of the summaries. When a range of LD/LC50 values was cited, the lowest value in the most relevant species (rodent or rabbit) and exposure scenario (e.g., 4-hour inhalation) was selected. In cases where the lowest LD/LC50 value was obtained using a non-traditional species or one so taxonomically removed from rodents (e.g., non-human primates) as to call into question the validity of a concordance analysis, preference was given to the next lowest LD/LC50 value in rodents or rabbits. LD/LC50 values obtained from sources that reported any uncertainty regarding data quality (e.g., inhalation studies in which maximum concentration was not achieved) were excluded from the analysis.

The resulting database is comprised of 429 agrochemical and biocidal active substances representing major product types (antimicrobials and other biocides, biochemicals, fungicides, herbicides, defoliants and plant growth regulators, insecticides, repellants and fumigants, vertebrate control agents, etc.). All LD/LC50 values were converted to regulatory classifications according to GHS criteria (UN, 2007). In cases where it was unclear whether a test atmosphere consisted of aerosols (solid or liquid), vapors, or a combination of the two, classifications were made according to GHS criteria for aerosols as recommended by Pauluhn et al. (1996). As above, substances with oral and dermal LD50 values in

excess of 2,000 mg/kg were considered for the purposes of this analysis as being “non-classified” for acute toxicity. This analysis has purposely excluded substances already examined by Creton et al. (2010). In total, concordance assessments are based on 337 substances that have been tested by both oral and dermal routes, and 348 tested by both oral and inhalation routes. The substances used for the concordance assessments are listed in a supplementary data file on www.altexediton.org.

3 Results and discussion

3.1 Oral-dermal concordance The relationship between acute oral and dermal toxicity classifications is summarized in Tables 1 and 2. For chemicals, the overall concordance among oral and dermal LD50 classifications was 93.7% across 1,569 substances. The oral and dermal route concordance for non-classified substances is 100%. The dermal test resulted in a more severe classification in only one instance (0.06% – classified as orally hazardous but dermally toxic), while the oral test yielded positive classifications for 98 substances (6.2%) that would have been underclassified by a dermal test alone. Of these, 88 were classed as hazardous, nine as toxic, and one as highly toxic.

For pesticides, the overall oral-dermal concordance was 54% across 337 substances (Tab. 2). For an additional 148 substances (43.92%) the oral test yielded more severe classifications, while the dermal test proved to be more sensitive in six cases (1.78%). Had the oral LD50 value alone been used as a basis for classification, the pesticides Furfural, Kelevan, Methylisothiazolinone, Mirex and Sodium Cyanide would have been underclassified by a single category, while Dowicil®CTAC (classified dermally as GHS Category 3) would have been unclassified. In the case of Sodium Cyanide (classified orally as GHS Category 2 and dermally as Category 1), the underclassification appears to be GHS-specific, i.e., the oral LD50 of 7.5 mg/kg is just above the 5 mg/kg threshold for inclusion in Category 1. This discordance would not have occurred under the former DSD scheme or if the US EPA or CPSC schemes had been used (Fig. 1), with their Category 1 thresholds of 25 mg/kg and 50 mg/kg, respectively. Furthermore, from a hazard-labeling standpoint (relevant for the protection of workers who may be directly exposed to pesticidal active substances), classification in GHS Category 2 as opposed to Category 1 has no impact on label signal word or hazard statement. For Furfural, Kelevan and Methylisothiazolinone (all oral Category 3/dermal Category 2), the signal word “danger” would still be present for all substances, although the hazard statement would be downgraded from “fatal in contact with skin” to “toxic in contact with skin” for a Category 3 classification. For Mirex (oral Category 4/dermal Category 3), both signal word and hazard statement would be downgraded, i.e., from “danger” to “warning” and from “toxic” to “harmful,” respectively. Dowicil®CTAC is the only substance that would have been overlooked entirely based on oral data (and a 2,000 mg/kg limit dose), whereas dermal results would have led to a GHS Category 3 classification and concomitant “danger” and “toxic” label statements, with advice that workers wear chemical-resistant gloves for open-pouring of the end-use product (EPA, 2007). The reasons behind the relatively greater sensitivity of the dermal route in this case are not clear at this time, in part due to the lack of dermal penetration studies in the database. Dowicil is considered to be a non-sensitizer based on a guideline study in guinea pigs. In a rabbit dermal irritation study, Dowicil produced only a slight edematous reaction on intact skin, resulting in a “slight irritant” label under the US EPA pesticide classification scheme; however, under the EU CLP scheme, the substance would not be considered a dermal irritant (EPA, 2004; OJ, 2008). The magnitude of the discordance in acute systemic classification results for Dowicil appears, to some extent, also to be classification scheme-specific, i.e., it would not have been as pronounced under the US pesticides scheme.

On the whole, these analyses illustrate the limited value of acute systemic toxicity testing *via* the dermal route for the purpose of classification and labeling, which is the primary driver for such studies (Seidle et al., 2010), thus calling into question the appropriateness of regulations that continue to require redundant

dermal route testing when oral data are already available. The extent to which dermal acute data provide added value for the regulation of formulated preparations/articles is also questionable (Thomas and Dewhurst, 2007) and warrants further investigation.

Tab. 1: Concordance among oral and dermal route acute toxicity classifications for industrial chemicals in the EU New Chemicals Database according to the former EU DSD scheme

	ORAL			
DERMAL	**NC**	**Xn**	**T**	**T+**
NC	1460	88	9	1
Xn	0	10	0	0
T	0	1	0	0
T+	0	0	0	0

[a] NC = no classification required; Xn = harmful; T = toxic; T+ = very toxic

Tab. 2: Concordance among oral and dermal route acute toxicity classifications for pesticide active substances according to the GHS (without the optional Category 5)

	ORAL				
DERMAL	**NC**	**4**	**3**	**2**	**1**
NC	154	96	32	2	2
4	0	6	4	2	0
3	1	1	9	6	2
2	0	0	3	4	2
1	0	0	0	1	10

Tab. 3: Distribution of substances listed in the EU New Chemicals Database through June 2008 with acute oral and dermal toxicity data according to REACH tonnage bands

	All substances registered in NCD (June 2008)	Substances with oral and dermal toxicity data (June 2008)
Substances manufactured or imported in quantities ≥1 tpa	25%	41%
Substances manufactured or imported in quantities ≥10 tpa	43%	53%

In the context of chemical regulation in the EU, Table 3 shows the distribution of substances registered in the NCD through June 2008 according to the two levels of information requirements established by REACH for acute systemic toxicity, i.e., annual production volume ≥1 metric ton (Annex VII) and annual production ≥10 metric tons (Annex VIII). Based on their production volume, 25% of all substances registered in the NCD fall within the REACH Annex VII requirement for a single-route (usually oral) acute toxicity study, while 43% of substances fall within the Annex VIII requirement for a second acute study (dermal or inhalation, depending on the nature of the substance and the likely route of human exposure). It is significant to note the relative prevalence of high-tonnage substances in the NCD, as well as the fact that nearly half of these (47%) appear not to possess acute toxicity data for more than one exposure route. Thus, a regulatory decision to waive the requirement for an acute dermal study for the estimated 10,000 existing substances that will be subject to REACH Annex VIII data requirements could potentially spare a large number of animals (assuming that dermal testing has not already been carried out, or that an inhalation study is not simply substituted in place of the dermal test; see 3.2. for additional discussion of this point).

With regard to pesticides, we note that at the time of this writing, EU data requirements for agrochemicals are undergoing revision, as are regulations governing the registration of biocides in the EU and antimicrobial pesticides in the US. A review and reconsideration of data requirements for dermal acute systemic toxicity would therefore be both timely and warranted in these regions and elsewhere. In relation to formulated pesticide products, the EU's longstanding acceptance of "classification by calculation", i.e., pursuant to Annex II of the former Directive 1999/45/EC, which has recently been replaced by Regulation (EC) No 1272/2008, could obviate the conduct of redundant in vivo testing of formulations comprised of well characterized active substances and other ingredients (OJ, 1999; 2008).

3.2 Oral-inhalation concordance The relationship between acute oral and inhalation toxicity classifications is summarized in Tables 4 and 5. For chemicals, the overall concordance among oral LD50 and inhalation LC50 classifications was 71.8% across 71 substances. The oral test resulted in a more severe classification in eight cases (11.3%, classified as orally hazardous but unclassified by the inhalation route), while the inhalation test yielded positive classifications for nine substances (6.2%) that would have been unclassified based on oral findings, and a further three substances (4.2%) that would have been underclassified by one or more DSD categories.

For pesticides, the oral-inhalation concordance was markedly lower: 24.12% across 348 substances classified according to GHS criteria (Tab. 5). For an additional 23.85% of substances, the oral test resulted in a more severe classification, whereas inhalation data led to a more severe classification for 51.72% of substances examined. Active substances classified as GHS Category 1 *via* inhalation but unclassified orally include Acequinocyl, Ammonium Thiosulfate, Bifenox, Bromacil, Bupirimate, Buprofezin, Clofencet, Cyflufenamid, Dichlobenil, Diflufenzopyr, Disodium tetraborate, Mesotrione, Metrafenone, Pine Oil, Piperonyl Butoxide, and Sintofen. Further investigation is needed to identify the physico-chemical (reactivity, particle size, vapor pressure, solubility), kinetic, metabolic, and other factors that underlie differences seen in acute toxic responses following oral and inhalation exposure (Pauluhn et al., 1996). It is possible, however, that at least some of the observed discordance could be an artifact of the classification system itself. As illustrated in Figure 3, criteria for making acute inhalation classifications differ considerably among countries and sectors. Differences include both the LC50 thresholds that define individual classification categories, as well as the fact that some systems establish separate criteria for aerosols (solid and liquid) *versus* vapors and gases while others do not. For example, the LC50 dose range for an inhalation Category 1 classification under the US pesticides scheme is quite narrow, i.e., up to 0.05 mg/l for all substances, regardless of their physical state. The same range is used under the GHS for dusts and mists; however, for vapors the upper threshold for a Category 1 classification is tenfold

higher, i.e., 0.5 mg/l, whereas the US pesticides scheme has established this dose level as the upper threshold for a Category II classification. Similarly, the dose range for a GHS Category 2 classification for vapors is identical to the US pesticide Category III, while vapors classified as Category IV under the US scheme would be classified as Category 3 under the GHS. Differences of comparable magnitude also would be evident if GHS classifications were compared against those of the former EU DSD scheme. Thus, depending on the classification scheme used, physical state of the substance in question and whether it is deemed to be predominantly mist or vapor over the course of a test, acute inhalation classifications could easily diverge by at least one severity category among GHS, DSD and EPA pesticide schemes. Additionally, there appears to be an inherent bias toward more severe classifications *via* the inhalation route, particularly under the GHS, which could provide some explanation for the low oral-inhalation concordance seen for pesticides.

Tab. 4: Concordance among oral and inhalation route acute toxicity classifications for industrial chemicals in the EU New Chemicals Database according to the former EU DSD scheme

	ORAL			
INHALATION	**NC**	**Xn**	**T**	**T+**
NC	50	8	0	0
Xn	4	1	0	0
T	3	1	0	0
T+	2	1	1	0

[a] NC = no classification required; Xn = harmful; T = toxic; T+ = very toxic

Tab. 5: Concordance among oral and inhalation route acute toxicity classifications for pesticide active substances according to the GHS (without the optional Category 5)

	ORAL				
INHALATION	**NC**	**4**	**3**	**2**	**1**
NC	36	23	17	2	3
4	53	38	17	6	8
3	29	17	7	4	2
2	23	17	7	3	1
1	16	11	4	3	0

From a regulatory perspective, REACH requires that all substances produced or marketed in the ≥10 tpa range in Europe must possess acute toxicity data for two exposure routes. Assuming that all or most dermal route testing could be waived, inhalation would become the new default second route by process of elimination. However, in light of the reasonably high oral-inhalation concordance and sensitivity of the

oral test for chemicals (83.1% when taken together), albeit across a limited data set, there may be value in exploring whether opportunities exist for expansion or refinement of existing criteria for waiving an inhalation study requirement under REACH (OJ, 2007; ECHA, 2008) and other international chemical regulatory schemes. Criteria currently specified in REACH technical guidance include knowledge of local toxicity, low volatility (i.e., vapor pressures <1 x 10-5 and <1 x 10-4 for indoor and outdoor uses, respectively), molecular weight, particle size (particles larger than 100 µm are less likely to be inhalable), mass median aerodynamic diameter, water solubility, reactivity, and ability to generate a stable test atmosphere (ECHA, 2008; Pauluhn et al., 1996). Waiving would not be envisioned in the case of highly acutely toxic substances for which derivation of an acute DNEL or AEGL is necessary. It may also be prudent to re-examine classification criteria for acute inhalation toxicity (i.e., LC50 cut-offs between categories) to verify the consistency of severity categories across exposure routes (e.g., that the severity of effects seen in an inhalation Category 2 are reasonably comparable to those of oral or dermal classifications of the same magnitude).

4 Conclusions and recommendations

Our analysis provides further, compelling evidence that dermal acute systemic toxicity data almost never drive regulatory classification and labeling decisions in the chemicals, agrochemicals, or biocides sectors. Thus, their contribution to the protection of consumers and workers would appear to be marginal at best and insufficient to warrant the routine conduct of redundant, lethal *in vivo* studies. We therefore recommend the following:

1. Dermal acute systemic testing should not be required for substances that are non-classified by the oral route, since the data clearly show that the oral route is almost always more sensitive, and the concordance among oral and dermal routes for non-classified substances is 100% for industrial and agrochemicals and 99.4% for biocides. Relevant national/regional data requirements for all three classes of substances should be revised accordingly (i.e., deleted or downgraded to conditional requirements) together with applicable implementing guidance.

2. Before a new dermal acute toxicity study is carried out, an *in vitro* dermal absorption/penetration study (OECD, 2004) should be conducted to assess the likely magnitude and rate of dermal bioavailability.

3. Data requirements prescribing acute dermal systemic testing of formulated agrochemical and biocidal products and well-characterized mixtures should be reconsidered in light of data presented and the established and conservative practice of classification by calculation.

4. It is important to avoid the situation under REACH whereby inhalation becomes the default second route for acute testing for substances in the ≥10 tpa tonnage band (by process of elimination if dermal testing is discontinued). Opportunities for refinement or expansion of existing criteria for adaptation or waiving of acute inhalation study requirements for chemicals should be fully explored.

5. Further investigation is needed to identify the physicochemical, kinetic, metabolic, and other factors that underlie differences seen in acute toxic responses following oral and inhalation exposure.

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Supplementary material for: Examining the Regulatory Value of Multi-route Mammalian Acute Systemic Toxicity Studies

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Table A: Pesticide active substances used in oral-dermal concordance assessment

2,2-dibromo-3- nitrilopropionamide
2-[(Hydroxymethyl)-amino]etanol
2-Mercaptobenzothiazole (zinc salt)
2-phenylphenol
4-aminopyridine
4-Chlorophenoxy-acetic Acid
4,4-Dimethyloxazolidine
Acetamiprid
Acibenzolar-s-methyl
Acrinathrin
Acrolein
Adbac
Aldrin
Aliphatic Alkyl Quaternaries
Aliphatic Solvents
Alkyl amine hydrochloride
1-(2-hydroxyethyl)-2-alkyl-2-imidazoline
Alkyl trimethylenediamines
Alkylbenzene Sulfonates
Allethrin
Aluminum phosphide
Alphachloralose
Ametryn
Aminopyralid
Ammonium Thiosulfate
Ancymidol
Asulam sodium
Azadioxabicyclooctane
Barium metaborate
Benzisothiazoline-3-one
Benzoic acid
Bifenazate
Bifenthrin
Bioallethrin
Biobor
Bis(trichloromethyl) sulfone
Bitertanol
Boric acid
Boric oxide
Boscalid
Brodifacoum
Bromacil
Bromadiolone
Bromethalin
Bromohydroxy- acetophenone
Bromoxynil heptanoate
Bromoxynil octanoate
Bupirimate
Busan 77
Butralin
Butylate
cacodylic acid
calcium acid methanearsonate
Carbetamide
Carboxin
Chlorantraniliprole
Chlordane
Chlordecone
Chlordimeform
Chlorflurenol
Chlorhexidine diacetate
Chrlorinated isocyanurates
Chlormequat
p-Chloro-m-cresol
Chlorohydrin-alpha
Chloroneb
Chlorophacinoneq
Chloroxylenol
Chlorsulfuron
Clethodim
Clofencet
Clofentezine
Cloransulam-methyl
Clothianidin
Coumaphos
Coumatetralyl
Creosote oil (P1/P13 fraction)
Cryolite
Cyanamide
Cyazofamid
Cycloxydim
Cycloate
Cyflufenamid
Cymoxanil
Cyazofamid
Cyclanilide
Cyhalothrin-lambda
Cyproconazole
Cyprodinil
Dazomet
DEET
Denatonium benzoate
dibromodicyanobutane

Dicamba
Dichlobenil
Dichlorobenzoic acid methylester
Diclofop
Dicloran
Dichlorofluanid
Dicofol
Dicrotophos
Didecyldimethylammonium
chloride
Dieldrin
Diethofencarb
Difenacoum
Difenoconazole
Difenzoquat
Difethialone
Diflubenzuron
Diflufenzopyr
Diiodomethyl p-tolyl sulfone
Dimethipin
Dimethoate
Dimethoxane
Diphacinone
Diphenylamine
Dipropylene Glycol
Disodium octaborate tetrahydrate
Disodium cyanodithioimidocarbonate
disodium methanearsonate
Disodium tetraborate
Disulfoton
Dithianon
Dodine
Dowicil®CTAC
Endrin
EPTC
Esbiol
Ethalfluralin
Etofenprox
Etridiazole
Fenamidone
Fenazaquin
Fenbuconazole
Fenbutatin oxide
Fenoxycarb
Flonicamid
Florasulam
Fluazifop
Flubendiamide
Flucarbazone-sodium
Flufenoxuron
Flumetralin
Fluometuron
Fluopicolide
Fluroxypyr
Fluquinconazole
Flurochloridone
Fluthiacet-methyl
Foramsulfuron
Furfural
Gibberellic Acid
Glufosinate ammonium
Glutaraldehyde
Grotan
Guazatine
Heptamaloxyloglucan
Hexadecadienol acetates
Hexazinone
Hexythiazox
Hydramethylnon
Hyrdogen peroxide
Hydroxyethyl octyl sulfide
Hydroxypropyl
methanethiosulfonate
Imazaquin
Imiprothrin
Indoxacarb
Iodine
Iodomethane
IPBC
Ipconazole
Iprovalicarb
Iron salts
Isobenzan
Isoxaben
K-HDO
Kelevan
Laminarin
Lenacil
Lithium hypochlorite
Lithium perfluorooctane sulfonate
Macleaya extract
Magnesium phosphide
Mandipropamid
Mepiquat
Meptyldinocap
Mesotrione
Mesosulfuron-methyl
Metaldehyde
Metam-sodium
Metamitron
Metazachlor
Methoprene
Methyl nonyl ketone
Methylneodecanamide-N
Methyl Parathion
Methylene bis(thiocyanate)
Methylisothiazolinone
Metofluthrin

Metolachlor
Metolachlor-S
Metosulam
Mirex
Mitin FF
monosodium methanearsonate
Myclobutanil
Nabam
Naphthalene
Naphthaleneacetic acid (acetamide)
Naphthaleneacetic acid (ethyl ester)
Naptalam Sodium
Niclosamide
Nicosulfuron
Nicotine
Nitrapyrin
norflurazon
Novaluron
Nuosept 145®
o-benzyl-chlorophenol
OBPA
octhilinone
Sulfonated oleic acid, sodium salt
Organic esters of phosphoric acid
Orthosulfamuron
Oryzalin
Oxadiazon
Oxyfluorfen
p-dichlorobenzene
Paclobutrazol
Paranitrophenol
Pebulate
Pencycuron
Pentachloronitrobenzene
Pentachlorophenol
Periplanone B
Permethrin
Phorate
Picaridin
Picloram
Picolinafen
Pine oil
Pinoxaden
Piperalin
Piperonyl Butoxide
Polybutene
polyhexamethylenebiguanide
Potassium hydrogen carbonate
Potassium iodide
Potassium peroxymonosulfate
sulfate
Potassium thiocyanate
Prochloraz
Propachlor
Propanil
Propaquizafop
Propargite
Polypropylene Glycol
Prometon
Prometryn
Propachlor
Propanil
propargite
Propetamphos
Propoxur
Proquinazid
Putrescine
Pynamin Forte
Pyrasulfotole
Pyridaben
Pyridalyl
Pyriproxyfen
Pyroxsulam
Quinmerac
Quizalofop-P-tefuryl
Resmethrin
Rotenone
S-Kinoprene
Sabadilla Alkaloids
Sethoxydim
Siduron
Silver (sildate)
Silthiofam
Sintofen
Sodium Acifluorfen
Sodium chlorate
Sodium Cyanide
Sodium diacetate
Sodium Fluoride
Sodium Fluoroacetate
Sodium U-nitroguaiacolate
Sodium o-nitrophenolate
Sodium p-nitrophenolate
Sodium Omadine
Spirotetramat
Starlicide
Strychnine
Sulfentrazone
Sulfur
sumithrin
Tau-fluvalinate
TCMTB
Tebufenozide
Tebufenpyrad
Tebuthiuron
Teflubenzuron
Tefluthrin
Terbacil

Terbufos
Tembotrione
Terbuthylazine
Tetrachlorvinphos
Tetradifon
Tetramethrin
Thallium (I) sulfate
Thiacloprid
Thiazopyr
Thidiazuron
Thiobencarb
Thiram
Topramezone
Triadimefon
Triallate
Triazoxide
Tribufos
Trichloromelamine
Triclosan
Tridecenyl Acetates
Triflumizole
Triflumuron
Triflusulfuron
Triforine
Triphenyltin Hydroxide
tris(hydroxymethyl)nitromethane
Xylenol
(Z)-9-Tricosene
Zinc Phosphide
Zinc oxide
Zeta-cypermethrin

Table B: Pesticide active substances used in oral-inhalation concordance assessment

1,3-dichloropropene
2,2-dibromo-3- nitrilopropionamide
2-[(Hydroxymethyl)-amino]etanol
4-aminopyridine
4-Chlorophenoxy-acetic Acid
4,4-Dimethyloxazolidine
Abamectin
Acequinocyl
Acetamiprid
Acetochlor
Acibenzolar-s-methyl
Aclonifen
Acrinathrin
Acrolein
Adbac
Aliphatic Alkyl Quaternaries
Aluminum phosphide
Alphachloralose
Ammonium Thiosulfate
Ancymidol
Asulam sodium
Azadioxabicyclooctane
Barium metaborate
Benfluralin
Benfuracarb
Bensulfuron
Benthiavalicarb
Benzisothiazoline-3-one
Benzoic acid
Bifenazate
Bifenox
Bifenthrin
Bioallethrin
Bispyribac sodium
Bis(trichloromethyl) sulfone
Bitertanol
Boric acid
Boric oxide
Boscalid
Brodifacoum
Bromacil
Bromadiolone
Bromethalin
Bromohydroxy- acetophenone
Bromoxynil heptanoate
Bromoxynil octanoate
Bromuconazole
Bupirimate
Buprofezin
Busan 77
Butralin
Butylate
Cacodylic acid
Cadusafos
Calcium acid methanearsonate
Captan
Carbaryl
Carbetamide
Carbofuran
Carbosulfan
Carboxin
Chlorantraniliprole
Chlordane
Chlorhexidine diacetate
Chloridazon
Chlordimeform
Chlorflurenol
p-Chloro-m-cresol
Chlorohydrin-alpha
Chlorophacinoneq
Chlorsulfuron
Clodinafop-propargyl
Clofencet
Clofentezine
Cloransulam-methyl
Coumaphos
Coumatetralyl
Creosote oil (P1/P13 fraction)
Cyanamide
Cyazofamid
Cycloxydim
Cyflufenamid
Cymoxanil
Cyprondinil
Cyazofamid
Cyclanilide
Cycloate
Cyhalofop-butyl
Cyhalothrin-lambda
Cymoxanil
Cypermethrin
Cyproconazole
Cyprodinil
Dazomet
DEET
Denatonium benzoate
dibromodicyanobutane
Dicamba
Dichlobenil
Dichlorobenzoic acid methylester
Dicloran
Dichlorofluanid
Dicofol
Dicrotophos

Dieldrin
Difenacoum
Difenoconazole
Diflufenzopyr
Diiodomethyl p-tolyl sulfone
Dimethenamid-P
Dimethipin
Dimethoate
Dimethachlor
Dimethomorph
Dimethoxane
Dimoxystrobin
Diphacinone
Dipropylene Glycol
Disodium cyanodithioimidocarbonate
disodium methanearsonate
Disodium octaborate tetrahydrate
Disodium tetraborate
Dithianon
Diuron
Dodine
Dowicil®CTAC
Endrin
Epoxiconazole
EPTC
Esbiol
Ethalfluralin
Ethephon
Etofenprox
Fenamidone
Fenamiphos
Fenazaquin
Fenbuconazole
Fenbutatin oxide
Fenoxaprop
Fenoxycarb
Fenpropidin
Fenpropimorph
Fenpyroximate
Fipronil
Flonicamid
Florasulam
Fluazifop
Fluazinam
Flubendiamide
Flucarbazone-sodium
Flufenoxuron
Flumetralin
Fluometuron
Fluoxastrobin
Fluroxypyr
Fluquinconazole
Flurochloridone
Fluthiacet-methyl
Flutolanil
Folpet
Foramsulfuron
Formetanate
Fosetyl-AL
Fuberidazole
Furfural
Gibberellic Acid
Glufosinate ammonium
Glutaraldehyde
Grotan
Guazatine
Heptamaloxyloglucan
Hexazinone
Hexythiazox
Hydrogen peroxide
Hydroxypropyl
methanethiosulfonate
Hymexazol
Imazaquin
Imiprothrin
Iodine
Ioxynil octanoate
IPBC
Ipconazole
Iprovalicarb
Iron salts
Isobenzan
Isoxaben
K-HDO
Kelevan
Laminarin
Lenacil
Lithium perfluorooctane sulfonate
Macleaya extract
Malathion
Mepiquat
Mesotrione
Mesosulfuron-methyl
Metaldehyde
Metamitron
Metconazole (cis:trans)
Methiocarb
Methoprene
Methyl bromide
Methyl nonyl ketone
Methylneodecanamide-N
Methylene bis(thiocyanate)
Methylisothiazolinone
Metofluthrin
Metolachlor-S
Metosulam
Metrafenone
Metribuzin

Metsulfuron-methyl
Mirex
Mitin FF
Monosodium methanearsonate
Myclobutanil
Nabam
Naphthalene
Naphthaleneacetic acid (ethyl ester)
Naptalam Sodium
Niclosamide
Nicotine
Nitrapyrin
norflurazon
Novaluron
o-benzyl-chlorophenol
Organic esters of phosphoric acid
Orthosulfamuron
Oryzalin
Oxadiazon
Oxydemethon-methyl
Oxyfluorfen
p-dichlorobenzene
Paclobutrazol
Paranitrophenol
Pebulate
Penconazole
Pencycuron
Pentachloronitrobenzene
Permethrin
Phorate
Phosalone
Phosmet
Picloram
Pine oil
Piperalin
Primicarb
Pinoxaden
Piperonyl Butoxide
Pirimiphos-methyl
Polybutene
polyhexamethylenebiguanide
Potassium hydrogen carbonate
Potassium iodide
Potassium peroxymonosulfate
sulfate
Potassium thiocyanate
Propachlor
Propamocarb
Propaquizafop
Propiconazole
Prometon
Propachlor
Propanil
propargite
Propetamphos
Propoxur
Propylene Oxide
Proquinazid
Prosulfocarb
Prothioconazole
Putrescine
Pynamin Forte
Pyrasulfotole
Pyridaben
Pyridalyl
Pyrimethanil
Pyroxsulam
Quinmerac
Quinoclamine
Quizalofop-P-tefuryl
Resmethrin
Rimsulfuron
Rotenone
S-Kinoprene
Sabadilla Alkaloids
Sethoxydim
Siduron
Silver (sildate)
Silthiofam
Sintofen
Sodium Acifluorfen
Sodium Fluoride
Sodium Fluoroacetate
Sodium 5-nitroguaiacolate
Sodium o-nitrophenolate
Spirodiclofen
Spirotetramat
Starlicide
Strychnine
Sulcotrione
Sulfentrazone
Sulfur
sumithrin
Tebuconazole
Tebufenozide
Tebufenpyrad
Tebuthiuron
Tefluthrin
Tembotrione
Terbuthylazine
Tetradifon
Terbacil
Terbufos
Tetrachlorvinphos
Tetramethrin
Thallium (I) sulfate
Thiacloprid
Thiamethoxam

Thiazopyr
Thiodicarb
Thiram
Tolclofos-methyl
Tolyfluanid
Topramezone
Tralkoxydim
Triallate
Triadimefon
Triadimenol
Triazoxide
Tribenuron-methyl
Tribufos
Trichloromelamine
Triclopyr
Triclosan
Tridecenyl Acetates
Triflumizole
Triflumuron
Trifluralin
Triflusulfuron
Triforine
Trinexapac
tris(hydroxymethyl)nitromethane
Triticonazole
Vancide
Xylenol
(Z)-9-Tricosene
Zinc Phosphide
Zinc oxide