Retrospective Analysis of Acute Systemic Toxicity Tests for Pesticides

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Introduction

- The U.S. Environmental Protection Agency (EPA) requires in vivo acute toxicity testing to determine the potential systemic toxicity of pesticide products.
- These tests yield an LD50 value (dose expected to produce lethality in 50% of the animals tested). This value is used to determine hazard classifications, which specify labeling, personal protective equipment, and handling requirements to protect human health and the environment (Figures 1 and 2).
- One of the EPA-required tests assesses toxicity of substances absorbed through the skin (acute dermal systemic toxicity). In this study, we evaluated data from acute oral and dermal systemic toxicity tests to determine if oral LD50 values could be used to assign hazard classifications for dermal toxicity. Using oral toxicity test data to determine dermal hazard classification could reduce animal use for required acute systemic toxicity testing.
- We evaluated data for both pesticide formulations and active ingredients (AIs), and considered both the EPA hazard classification system and the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Hazard Classification

- **Figures 1** and **2** show EPA and GHS hazard classification schemes, respectively, including EPA personal protective equipment requirements.
    - The EPA requires labeling for any substance with a dermal or oral LD50 value less than or equal to 5000 mg/kg, and divides these into categories I, II, and III (EPA 2012).
    - The GHS provides labeling guidance for substances with dermal or oral LD50 values less than or equal to 2000 mg/kg, and divides these into categories 1, 2, 3, and 4 (UN 2015).
- Current procedures for acute dermal systemic toxicity testing, as described in test guidelines issued by EPA (OPPTS 870.1200; EPA 1998) and the Organisation for Economic Co-operation and Development (Test Guideline 402; OECD 1987), recommend using a minimum of 20 animals for the main test.

Figure 1 EPA Acute Oral and Dermal Hazard Categories

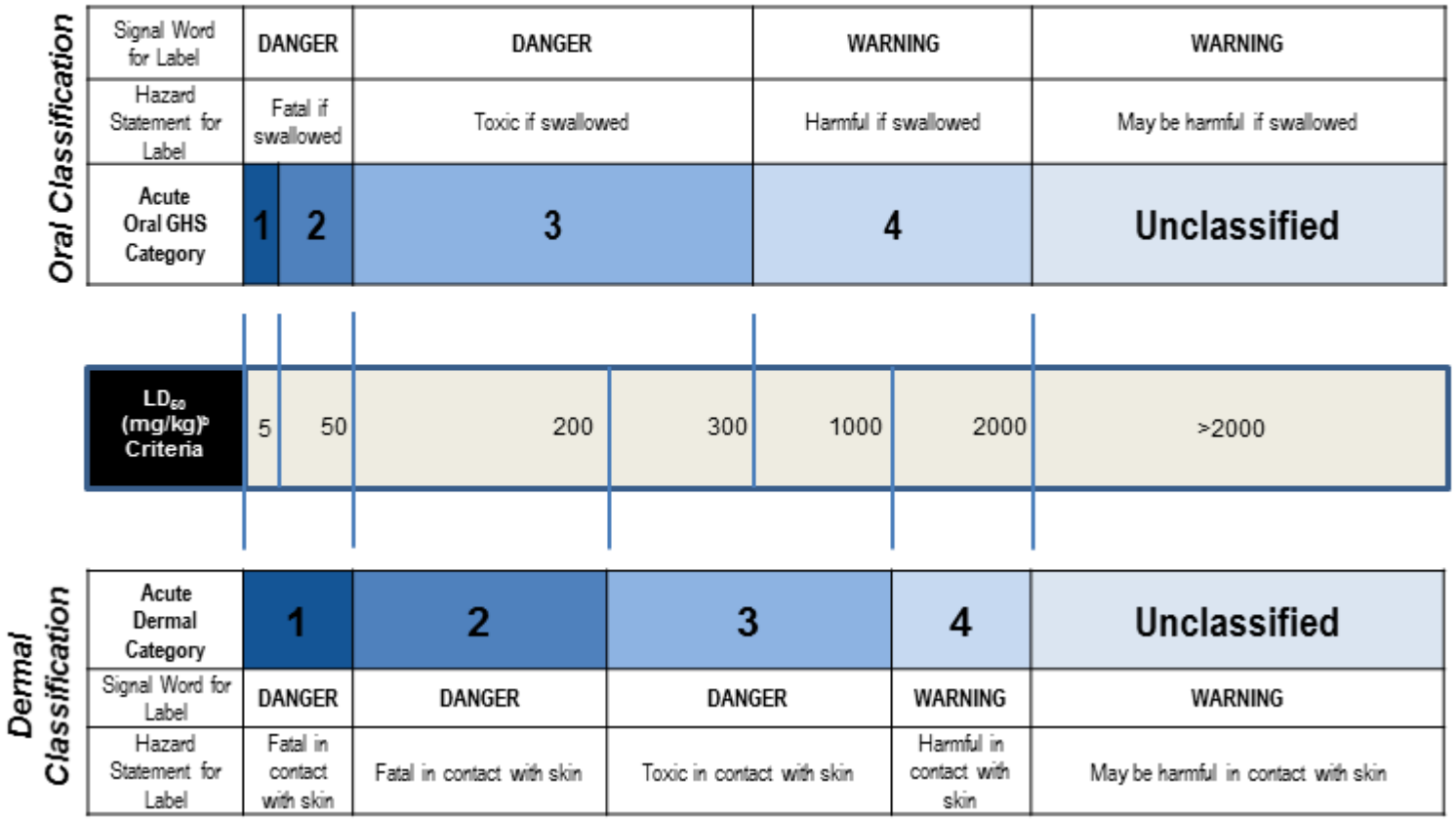
Oral Classification					
	Signal Word for Label	**DANGER-POISON**	**WARNING**	**CAUTION**	**CAUTION** (optional)
	Hazard Statement for Label	Fatal if swallowed	May be fatal if swallowed	Harmful if swallowed	NR or optionally "harmful if swallowed"
	EPA Oral Category	**I**	**II**	**III**	**IV**

LD_{50} (mg/kg)	50	200	500	2000	5000	>5000

Dermal Classification					
	EPA Dermal Category	**I**	**II**	**III**	**IV**
	Signal Word for Label	**DANGER-POISON**	**WARNING**	**CAUTION**	**CAUTION** (optional)
	Hazard Statement for Label	Fatal if absorbed through skin	May be fatal if absorbed through skin	Harmful if absorbed through skin	NR or optionally "harmful if absorbed through skin"
	Personal Protective Equipment	Coveralls worn over long-sleeved shirt and long pants; socks; chemical-resistant footwear; chemical-resistant gloves	Coveralls worn over short-sleeved shirt and short pants; socks; chemical-resistant footwear; chemical-resistant gloves	Long-sleeved shirt and long pants; socks; shoes; chemical-resistant gloves	Long-sleeved shirt and pants; socks; shoes

Abbreviations: EPA = U.S. Environmental Protection Agency; LD50 = dose expected to produce lethality in 50% of the animals tested; NR = not required.

Figure 2 GHS Acute Oral and Dermal Hazard Categories



Abbreviations: GHS = Globally Harmonized System of Classification and Labelling of Chemicals; LD50 = dose expected to produce lethality in 50% of the animals tested; Uncl. = unclassified.

Development of the Dataset

- We developed two databases of substances that had both rat acute oral toxicity data and rat acute dermal toxicity data available: one with 612 pesticide formulations and the other with 298 pesticide AIs.
- Data were obtained from:
  - EPA documents: Data Evaluation Reports (DERs), Reregistration Eligibility Decision (RED) documents, and contract research organization laboratory reports
  - Peer-reviewed publication on acute toxicity testing of chemicals (Creton et al. 2010)
  - Public toxicity databases
    - Hazardous Substances Data Bank (National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB)
    - European Chemicals Agency database (http://echa.europa.eu/information-on-chemicals/registered-substances)
- Data quality was evaluated according to Klimisch et al. 1997.
  - Code 1: Data reliable without restrictions
  - Code 2: Data reliable with restrictions
  - Code 3: Data not reliable
  - Code 4: Data not assignable
- Only data with reliability codes of 1 or 2 were included the in analyses.

Evaluation of the Dataset

- To determine if oral LD50 values could be used to assign hazard classifications for both oral and dermal toxicity, we evaluated the dataset of 612 pesticide formulations and 298 pesticide AIs for GHS and a subset of 588 pesticide formulations and 238 pesticide AIs for the EPA system.
  - The EPA classifications did not include 24 formulations and 60 AIs for which acute oral LD50 values were reported as >5000 mg/kg **and** acute dermal LD50 values were reported as >2000 mg/kg.
    - These substances were omitted because different limit tests were performed for the oral and dermal routes of exposure (oral LD50 >5000 and dermal LD50 >2000). Classification would be Category III (EPA) and unclassified (GHS). However, the EPA categorization could be an overestimation of hazard because the LD50 >2000 mg/kg might actually be >5000 mg/kg, which would enable its assignment to the less restrictive EPA Category IV.

- Both EPA and GHS oral and dermal hazard classifications were based on the LD50 value reported from the in vivo tests (see **Figures 1** and **2**). If more than one LD50 value was reported for a substance, the substance was classified based on the lowest LD50 value (resulting in a more restrictive classification).
- **Table 1** describes statistics we calculated to evaluate how well oral LD50 values could predict hazard classifications for dermal toxicity.

Table 1 Calculating Concordance, Overprediction, Underprediction, and Predictivity of Dermal Hazards When Using Acute Oral Hazard Classifications

Classification	Formula
Concordant Hazard Classification (based on total classifications)	Total C% = (C/C+O+U)*100
Overpredicted Hazard Classification (based on total classifications)	Total O% = (O/C+O+U)*100
Underpredicted Hazard Classification (based on total classifications)	Total U% = (U/C+O+U)*100
Predictivity (based on single oral classification)	Categorical P% = (C/C+O+U)*100

Abbreviations: C = number of concordant substances; O = number of overpredicted substances;
P = proportion of concordant dermal hazard category matches to an oral hazard category;
U = number of underpredicted substances.

Results

- Comparison of oral hazard classifications based on rat oral LD50 values with dermal hazard classifications based on rat dermal LD50 values are found in **Tables 2** and **3** (formulations), **Tables 4** and **5** (active ingredients), and **Figures 3** and **4**.
  - Concordance of oral and dermal hazard classification:
    - Formulations: 56% for the EPA classifications (**Table 2**) and 69% for the GHS classifications (**Table 3**)
    - Active Ingredients: 61% for the EPA classifications (**Table 4**) and 55% for the GHS classifications (**Table 5**)
  - Overprediction of dermal hazard:
    - Formulations: 44% for the EPA system and 30% for the GHS
    - Active Ingredients: 35% for the EPA system and 41% for the GHS
    - Overprediction means that, based on oral test results, the substance would be assigned to a hazard classification that is more severe than the one that would be assigned based on dermal test results.
  - Modified concordance was determined by combining concordant and overpredicted results.
    - Formulations: 99% for both the EPA system and the GHS
    - Active Ingredients: 96% for the EPA system and 97% for the GHS
    - These high values would provide a dermal hazard classification based on the oral hazard classification that would require personal protective equipment at least as protective as the classification determined from directly measuring the dermal toxicity.
  - Underprediction of dermal hazard:
    - Formulations: 1% for the EPA system and 1% for the GHS
    - Active Ingredients: 4% for the EPA system and 3% for the GHS
    - Underprediction means that, based on oral test results, the substance would be assigned to a hazard classification that is less severe than the one that would be assigned based on dermal test results.
  - Predictivity on a category-to category basis is reported in **Tables 2** and **3** (EPA) and **Tables 3** and **4** (GHS). Predictivity was highest for formulations for EPA dermal Category IV (99%; **Table 2**) and GHS dermal category Unclassified (99.5%; **Table 3**).
    - These classifications do not require hazard labeling.

Table 2 Classification of Pesticide Formulations Using EPA Oral Hazard Categories to Predict EPA Dermal Categories*

	EPA Oral Category I	EPA Oral Category II	EPA Oral Category III	EPA Oral Category IV	Total	Concordant	Overpredicted	Underpredicted
EPA Dermal Category I	1	0	0	0	1	100%	NA	0%
EPA Dermal Category II	0	5	3	1	9	56%	0%	44%
EPA Dermal Category III	0	26	116	0	142	82%	18%	0%
EPA Dermal Category IV	0	31	199	206	436	47%	53%	NA
Total	1	62	318	207	588	56%	44%	1%
Predictivity	100%	8%	36%	99%				

Abbreviations: EPA = U.S. Environmental Protection Agency.

*Excludes 24 substances with oral LD50 >5000 and dermal LD50 >2000 (limit tests)

Overpredicted hazard category

Concordant hazard category

Underpredicted hazard category

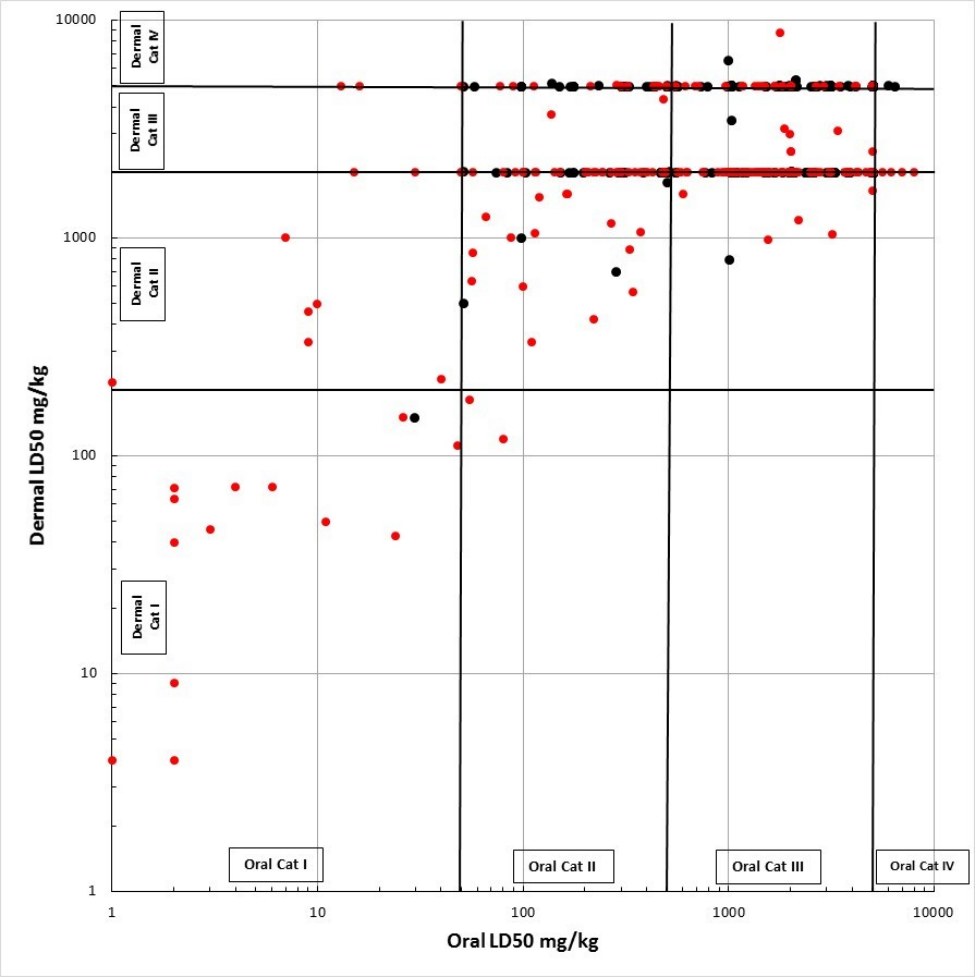
Table 3 Classification of Pesticide Formulations Using GHS Oral Hazard Categories to Predict GHS Dermal Categories

	GHS Oral Category 1	GHS Oral Category 2	GHS Oral Category 3	GHS Oral Category 4	GHS Oral Uncl	Total	Concordant	Overpredicted	Underpredicted
GHS Dermal Category 1	0	0	0	0	0	0	0%	NA	0%
GHS Dermal Category 2	0	1	0	0	0	1	100%	0%	0%
GHS Dermal Category 3	0	0	2	1	0	3	67%	0%	33%
GHS Dermal Category 4	0	0	1	3	2	6	50%	17%	33%
GHS Dermal Uncl	0	0	24	160	418	602	69%	31%	NA
Total	0	1	27	164	420	612	69%	30%	1%
Predictivity	0%	100%	7%	2%	99.5%				

Abbreviations: GHS = Globally Harmonized System of Classification and Labelling of Chemicals; Uncl = unclassified.

Overpredicted hazard category
Concordant hazard category
Underpredicted hazard category

Figure 3 Distribution of Oral vs. Dermal Data for Pesticide Formulations and Active Ingredients by EPA Hazard Category



● = pesticide formulations

● = pesticide active ingredients

Overlapping points not indicated.

Table 4 Classification of Pesticide Active Ingredients Using EPA Oral Hazard Categories to Predict EPA Dermal Categories*

	EPA Oral Category I	EPA Oral Category II	EPA Oral Category III	EPA Oral Category IV	Total	Concordant	Overpredicted	Underpredicted
EPA Dermal Category I	13	2	0	0	15	87%	NA	13%
EPA Dermal Category II	6	15	5	0	26	58%	23%	19%
EPA Dermal Category III	4	33	92	2	131	70%	28%	2%
EPA Dermal Category IV	2	11	27	26	66	39%	61%	NA
Total	25	61	124	28	238	61%	35%	4%
Predictivity	52%	25%	74%	93%				

Abbreviations: EPA = U.S. Environmental Protection Agency.

*Excludes 60 substances with oral LD50 >5000 and dermal LD50 >2000 (limit tests)

Overpredicted hazard category

Concordant hazard category

Underpredicted hazard category

Table 5 Classification of Pesticide Active Ingredients Using GHS Oral Hazard Categories to Predict GHS Dermal Categories

	GHS Oral Category 1	GHS Oral Category 2	GHS Oral Category 3	GHS Oral Category 4	GHS Oral Uncl	Total	Concordant	Overpredicted	Underpredicted
GHS Dermal Category 1	5	2	0	0	0	7	71%	NA	29%
GHS Dermal Category 2	3	3	2	0	0	8	38%	38%	24%
GHS Dermal Category 3	1	4	6	3	0	14	43%	36%	21%
GHS Dermal Category 4	0	1	6	3	3	13	23%	54%	23%
GHS Dermal Uncl	0	6	25	77	148	256	58%	42%	NA
Total	9	16	39	83	151	298	55%	41%	3%
Predictivity	56%	19%	15%	4%	98%				

Abbreviations: GHS = Globally Harmonized System of Classification and Labelling of Chemicals; Uncl = unclassified.

Overpredicted hazard category
Concordant hazard category
Underpredicted hazard category

Figure 4 **Comparison of Concordance, Overprediction, and Underprediction of Formulations and Active Ingredients Using EPA or GHS Classification Systems**

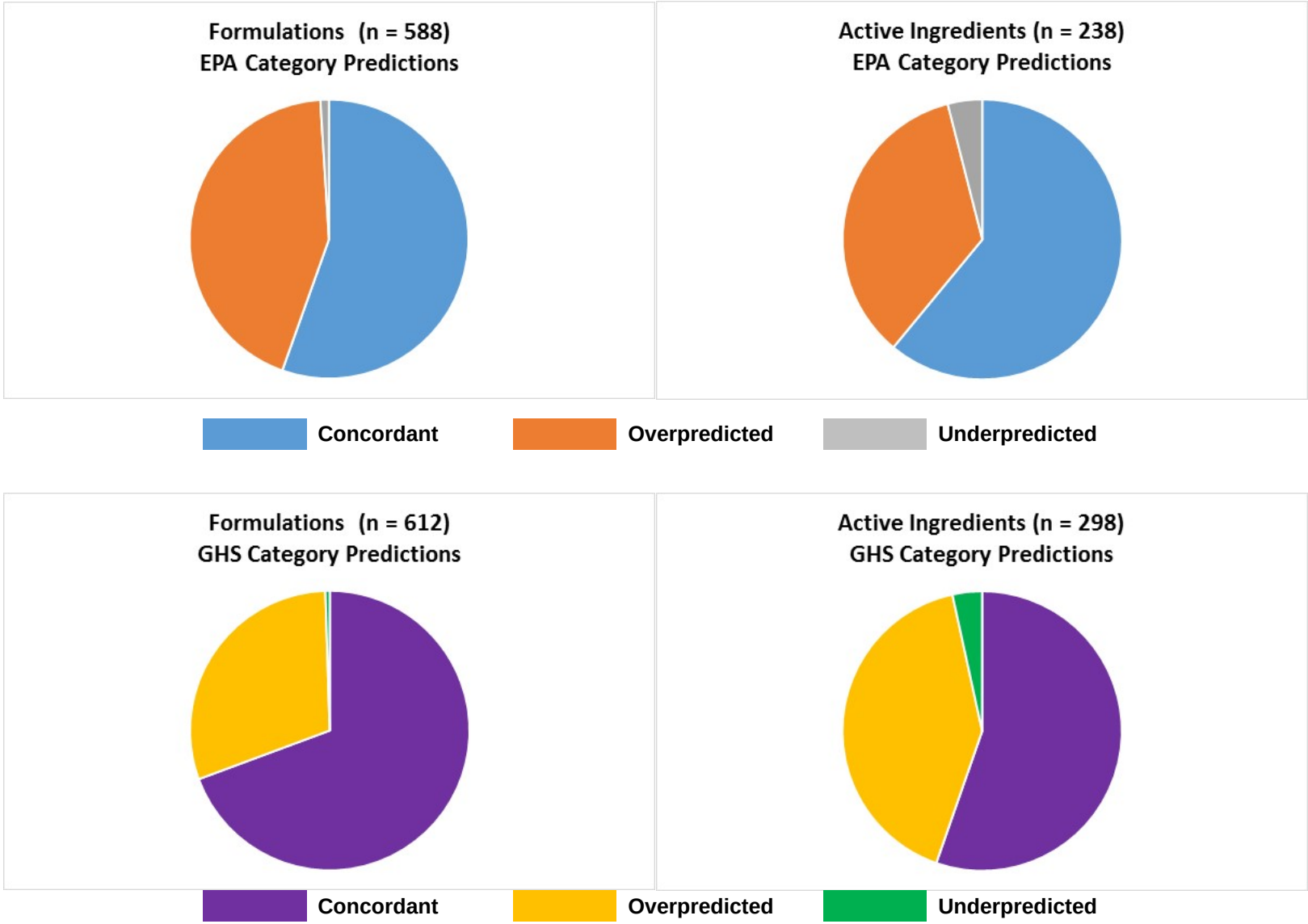


Table 6 Summary of Concordance, Overprediction, and Underprediction

Dataset	Concordant	Modified Concordance	Overpredicted	Underpredicted
EPA **Formulations** (n = 588)	56% (328/588)	99% (584/588)	44% (256/588)	1% (4/588)
GHS **Formulations** (n = 612)	69% (424/612)	99% (609/612)	30% (185/612)	1% (3/612)
EPA **Active Ingredients** (n = 238)	61% (146/238)	96% (229/238)	35% (83/238)	4% (9/238)
GHS **Active Ingredients** (n = 298)	55% (165/298)	97% (288/298)	41% (123/298)	3% (10/298)

Summary

- Our retrospective analysis of 612 formulations and 298 AIs used high-quality acute toxicity data (evaluated via Klimisch et al. 1997) obtained from EPA documents, a peer-reviewed publication, and public toxicity databases. The majority of the substances had oral and dermal hazard classifications in the least toxic categories:
  - EPA Categories III and IV – Formulations: 90% (oral), 98% (dermal)
  - EPA Categories III and IV – Active Ingredients: 71% (oral), 86% (dermal)
  - GHS Categories 4 and unclassified – Formulations: 95% (oral), 99% (dermal)
  - GHS Categories 4 and unclassified – Active Ingredients: 79% (oral), 90% (dermal)
- Concordance of oral and dermal hazard categories for formulations in the datasets was 56% for the EPA system and 69% for the GHS. Including the overprediction percentages with concordance (i.e., modified concordance) produced 99% modified concordance for the EPA subset and the GHS dataset.
- Predictivity of the least toxic hazard categories for the formulations datasets was 99% for the EPA system and the GHS.

Conclusions

- Many pesticide formulations and active ingredients could have the dermal hazard overstated if only oral LD50 values are used for predicting dermal hazard. However, this classification would provide for appropriate personal protective equipment for the handling of the pesticide formulations and active ingredients.
- The data show that it is unusual for a substance to be more toxic via the dermal route of exposure than the oral route. Very few of the substances in the database had acute dermal toxicity values that classified the substances as hazardous. Thus the underprediction of dermal hazard classification is very low and the modified concordance is high.
- Most of the underprediction of dermal hazard classification can be attributed to pesticides for which oral LD50 >5000 mg/kg and dermal LD50 >2000 mg/kg were reported. Exclusion of such formulations from the EPA analysis resulted in 99% predictivity for EPA dermal category IV.
- The predictivity for GHS unclassified substances was also 99% without excluding these substances because both oral and dermal classifications were unclassified (all LD50 values >2000 mg/kg). These analyses suggest that acute oral systemic toxicity data can be used to provide protective hazard classifications for acute dermal systemic toxicity for pesticide formulations and active ingredients, which would substantially reduce the number of animals used for acute systemic toxicity testing.

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