

## SAVING TWO BIRDS WITH ONE STONE: USING ACTIVE SUBSTANCE AVIAN ACUTE TOXICITY DATA TO PREDICT FORMULATED PLANT PROTECTION PRODUCT TOXICITY

SAMUEL K. MAYNARD,\* PETER EDWARDS, and JAMES R. WHEELER

Product Safety, Jealott's Hill International Research Centre, Syngenta, Bracknell, Berkshire, United Kingdom

(Submitted 21 January 2014; Returned for Revision 11 March 2014; Accepted 20 March 2014)

**Abstract:** Environmental safety assessments for exposure of birds require the provision of acute avian toxicity data for both the pesticidal active substance and formulated products. As an example, testing on the formulated product is waived in Europe using an assessment of data for the constituent active substance(s). This is often not the case globally, because some countries require acute toxicity tests with every formulated product, thereby triggering animal welfare concerns through unnecessary testing. A database of 383 formulated products was compiled from acute toxicity studies conducted with northern bobwhite (*Colinus virginianus*) or Japanese quail (*Coturnix japonica*) (unpublished regulatory literature). Of the 383 formulated products studied, 159 contained only active substances considered functionally nontoxic (median lethal dose [LD50] > highest dose tested). Of these, 97% had formulated product LD50 values of >2000 mg formulated product/kg (limit dose), indicating that no new information was obtained in the formulated product study. Furthermore, defined (point estimated) LD50 values for formulated products were compared with LD50 values predicted from toxicity of the active substance(s). This demonstrated that predicted LD50 values were within 2-fold and 5-fold of the measured formulated product LD50 values in 90% and 98% of cases, respectively. This analysis demonstrates that avian acute toxicity testing of formulated products is largely unnecessary and should not be routinely required to assess avian acute toxicity. In particular, when active substances are known to be functionally nontoxic, further formulated product testing adds no further information and unnecessarily increases bird usage in testing. A further analysis highlights the fact that significant reductions (61% in this dataset) could be achieved by using a sequential testing design (Organisation for Economic Co-operation and Development test guideline 223), as opposed to established single-stage designs. *Environ Toxicol Chem* 2014;33:1578–1583. © 2014 The Authors. Published on behalf of SETAC by Wiley Periodicals, Inc. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

**Keywords:** Avian toxicology   Ecological risk assessment   Ecotoxicology   Hazard/risk assessment   Pesticide regulation

## INTRODUCTION

Bird toxicity tests are required for the registration of plant protection product active substances (substances that provide the pesticidal mode of action) and in some regions also their formulated products (commercial product containing at least 1 active substance and coformulants such as solvents or dispersants designed for use in the field). Data from these tests can be required for classification, labeling, and/or risk assessment. Typically, bird acute oral (gavage) and long-term dietary reproduction studies are required for the active substance. In some regions, active substance short-term dietary studies are also required or may be triggered by acute toxicity values. Importantly, some regions also require avian acute oral studies for every formulated product in addition to the active substance data. Global data requirements for active substances and formulated products from selected regions (Europe, United States, Brazil, India, and China [1–6]) are summarized in Table 1.

In terms of animal numbers, long-term avian studies are the most animal intensive. However, the number of birds used for acute formulated product testing also is a matter of concern for animal welfare if these tests do not provide any routine benefit. Furthermore, active substances can be used in many different products for different uses and are often mixed with different partners. Where acute tests are required for each of these products, the number of studies and number of birds used can be considerable over the life cycle of an active substance [7].

Because bird acute active substance data are always available (Table 1), it has been suggested that simple calculations can be used to predict formulated product toxicity [8] and thus negate the need for formulated product-specific testing. Indeed, in Europe, the requirement to conduct formulated product bird acute oral studies was replaced by an assessment based on active substance data alone [6]. This approach has a strong rationale because, within a formulated product, the active substance is generally the toxicologically active component driving the overall toxicity of the product. Therefore, toxicity can be predicted reliably based on the toxicity of the active substances and their respective content in the formulated product. Conversely, for those active substances that are not toxic to birds (typically defined as those not causing mortality at a limit dose of 2000 mg active substance/kg body wt), dilution in a formulation is even less likely to lead to measurable toxicity (median lethal dose [LD50] < 2000 mg formulated product/kg body wt). These principles have been previously demonstrated to apply in regard to fish toxicity testing of active substances and their formulated products [9–11].

The bird acute oral toxicity test design has evolved from the initial methods described in 1982 by the US Environmental Protection Agency (EPA) 71-1 [12] and in 1996 by the Organisation for Economic Co-operation and Development (OECD) [13]. Currently, the test guidelines typically employed for birds are the USEPA Office of Chemical Safety and Pollution Prevention (OCSPP) 850.2100 [14] issued in 2012, a single-stage, 60-bird design, and OECD test guideline 223 issued in 2010 [15] a multistage sequential testing design. Both guidelines are designed to generate data that can be used to estimate both an LD50 and the slope of the dose–response relationship for acute oral toxicity. However, the 2 designs differ substantially, with the sequential

\* Address correspondence to sam.maynard@syngenta.com.  
Published online 26 March 2014 in Wiley Online Library  
(wileyonlinelibrary.com).  
DOI: 10.1002/etc.2590

Table 1. Comparison of the data requirements across the areas of most common global registration of active substances (a.s.) and formulated plant protection products (PPP)

Testing	Country									
	Europe		USA		Brazil		India		China	
	a.s.	PPP	a.s.	PPP	a.s.	PPP	a.s.	PPP	a.s.	PPP
Acute toxicity test	R	NR <sup>a</sup>	R	NR	R	R	R	R	R	R
Avian dietary toxicity test	NR	NR	R	NR	R	NR <sup>b</sup>	NR	NR	R	NR
Long-term reproduction test	R	NR	R	NR	R	NR	NR	NR	NR (R <sup>c</sup> )	NR

<sup>a</sup>Required when toxicity cannot be reliably predicted from the constituent active substances.

<sup>b</sup>Required if active substance LD50 ≤ 500 mg a.s./kg.

<sup>c</sup>Possibility for requirement in new updates to guidance.

R = required; NR = not required; LD50 = median lethal dose.

design (OECD test guideline 223) offering considerable benefits in terms of reducing the overall numbers of birds used and increasing the frequency of the generated dose response [16]. The number of birds used for bird acute oral toxicity tests is further explored using the dataset collected in the present study.

The present study aims to provide a quantitative, data-driven analysis to challenge the need for any avian acute oral toxicity tests with formulated plant protection products. We hope that a robust demonstration, based on pre-existing data, will lend support to a nontesting approach to formulated product evaluation in those regions and countries currently requiring these bird tests. This initiative originated from discussions at the United Kingdom's National Centre for Replacement, Refinement, and Reduction of Animals in Research ecotoxicology working group.

## MATERIALS AND METHODS

### Database construction

Data on LD50 were collected from the regulatory avian acute oral toxicity studies of 5 major agrochemical companies (Syngenta, Dow AgroSciences, Bayer CropScience, BASF, and Dupont). Data for formulated plant protection products and relevant active substances were compiled into a database. The data were selected in an unbiased manner (all available data for 2 companies, all products currently in use for 1 company, data from the past 15 yr for 1 company, and all available formulations containing more than 1 active substance for 1 company).

Formulated product toxicity data were restricted to studies conducted with 2 quail species: Japanese quail (*Coturnix japonica*) and northern bobwhite quail (*Colinus virginianus*). These 2 species are typically tested with formulated products. For active substances, the lowest LD50 values were used irrespective of species, because this was considered to be a suitably conservative approach; that is, predictions of formulated product toxicity are made from the most sensitive species tested with the active substance, although the measured formulated product toxicities may be from a less sensitive species.

Active substance LD50 data were used in conjunction with the content of the active substance in the formulation (% w/w) for each formulated plant protection product, to generate a predicted formulated product LD50 value by using a variation of Finney's harmonic mean calculation [8,17]

$${}^pLD50 = \frac{100}{\left(\frac{\%A}{LD_A} + \frac{\%B}{LD_B} + \dots\right)}$$

where  ${}^pLD50$  is the predicted formulated product LD50,  $LD_x$  is the LD50 of active substance  $x$ , and  $\%X$  is the percent of active

substance  $x$  in the formulated product (w/w). Using the equation in this way takes into account not only the additive toxicity of each active substance but also the dilution of that active substance and therefore its relative toxicity in the formulated product. For those formulations in which only a single active substance was present, the formula described above simply calculates a dilution of the LD50 relative to the content of active substance in the formulated product. In the present study, coformulants (e.g., solvents, dispersants) were assumed to be inert (no measurable toxicity) and were not included in the prediction calculation. When the measured formulated product LD50 was a greater than value (typically limit tests performed at >2000 mg formulated product/kg), the predicted formulated product LD50 value was also adjusted to this limit value if the prediction exceeded the limit. This was to avoid artifactual sensitivity differences.

### Analyses

**Predicted versus measured toxicity.** The database was evaluated to determine whether functionally nontoxic active substances (meaning in the present study those active substances with LD50 values greater than the highest tested dose) gave rise to functionally nontoxic formulated products (meaning in the present study those with experimentally derived LD50 values ≥ 2000 mg formulated product/kg). The relationship between predicted and measured toxicity was also evaluated graphically against a 1:1 relationship to determine those instances in which predicted LD50 values were or were not protective of LD50 values obtained experimentally. Predictions that gave a lower LD50 than that measured in a test and fall below the 1:1 line are more conservative than testing and thus were not of concern for this analyses. Predictions that gave a higher LD50 value than that measured in a test fall above the line of equality on the graph and are categorized into those falling between 2-fold, 5-fold, and 10-fold above the line of equality (see 1:2, 1:5, and 1:10 lines superimposed onto Figure 1). This simple measure allows the data to be categorized into those of concern and those that are covered by normal variability.

**Impact on vertebrate use.** The database was also used to calculate how many birds were used in the studies performed (control and treatments). Numbers were taken from the actual study reports but excluded any birds used in range-finding tests. Therefore, these are realistic measures, not assumptions derived from generic guideline requirements. This presented a unique opportunity to assess the true performance of the test guidelines (particularly in respect to OECD test guideline 223); previously, only simulations for the potential reduction in animal use have been possible.

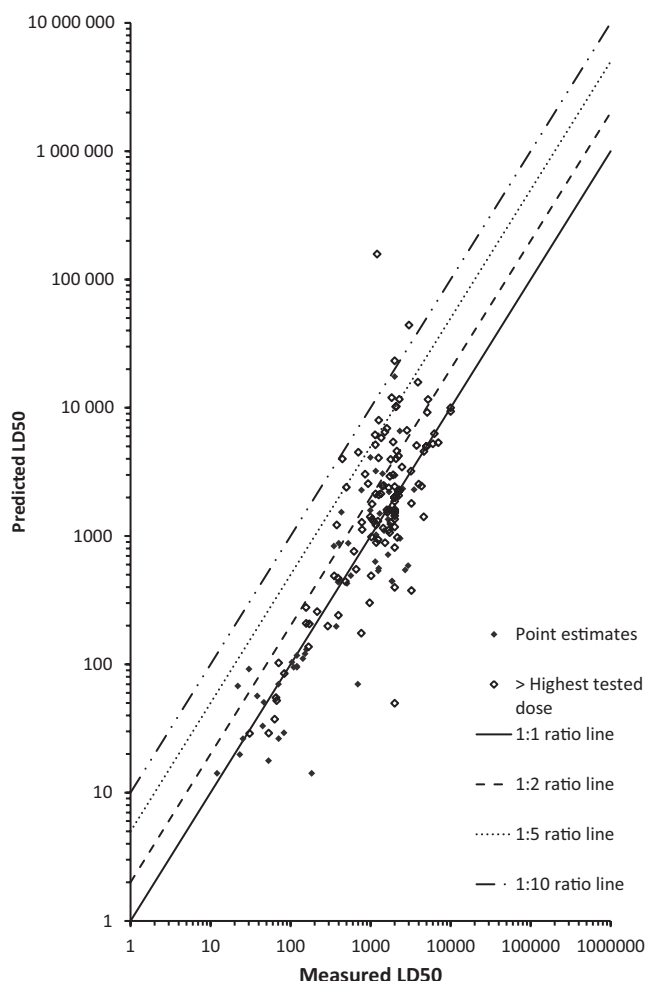


Figure 1. All 383 predicted median lethal dose (LD50) values plotted against measured LD50 values. Closed points designate data with only point estimated LD50 values, and open points designate data containing 1 or more LD50 values estimated as greater than the highest tested dose. Solid black line depicts 1:1 reference line (all points below this line demonstrate a prediction that produces a lower LD50 value [more toxic] than the measured value), and dotted and dashed lines depict 2-fold, 5-fold and 10-fold above the 1:1 line (demonstrating predictions that produce an LD50 value within 2-fold, 5-fold and 10-fold higher [less toxic] than the measured value).

## RESULTS

### Database description

The database contained data for 383 formulated products and their respective active substances. Data from each company were anonymized, and therefore exact numbers of active substances in the database could not be ascertained; however, each company provided data on 7 to 76 active substances. The database was considered representative of typical plant protection product types, covering anticipated uses, formulation types, and number of component active substances. Formulations containing 1 (35%), 2 (53%), 3 (10%), or 4 (2%) active substances were included. Formulation types were dominated by suspension concentrates (23%), emulsifiable concentrates (20%), and water-dispersible granules (17%) but also included flowable concentrates (10%), soluble concentrates (11%), and 20 other minor formulation types, which contributed the remaining 19% of the entries.

Of the formulated product studies collected, approximately two-thirds (67%) were conducted under the USEPA OCSPP 850.2100 guideline (or historic variants thereof; e.g., US Federal

Insecticide, Fungicide, and Rodenticide Act [FIFRA] subdivision E No. 71-1 or US Office of Prevention, Pesticides, and Toxic Substances 850.2100). Approximately one-fourth (23%) were conducted under the new OECD test guideline 223 guidance (sequential testing procedure), and the remainder (10%) were conducted according to various older, less common, or country-specific guidelines, which were termed "other."

### Predicted versus measured toxicity

Of the 383 formulated products, 166 contained only active substance data with unbounded ( $>$ ) LD50 values (termed functionally nontoxic active substances in the present study). Of these 166 formulated products, 93% (154) were also found to give rise to functionally nontoxic formulated products (measured LD50  $\geq$  2000 mg formulated product/kg body wt), indicating that functionally nontoxic active substances, when formulated, give rise to functionally nontoxic formulated products. The 12 instances in which this was not the case were investigated further. Seven of the 12 were found to contain an active substance that was tested only up to a maximum dose of 500 mg active substance/kg body weight (not the usual limit dose of 2000 mg active substance/kg body wt). Therefore, it is possible that the toxicity of this active substance was not properly characterized for the purposes of this analysis. In no instances was a formulated product containing this active substance found to have a measured LD50 value  $>$  2000 mg formulated product/kg body weight. If formulated products containing active substances that showed an LD50  $>$  500 mg active substance/kg body weight were removed as possibly containing a toxic active substance, then 97% (154/159) of formulated products containing only functionally nontoxic active substances result in functionally nontoxic formulated products. Only 3% (5/158) of the formulated products were found to have LD50 values below 2000 mg formulated product/kg body weight even though they contained only functionally nontoxic active substances.

Point-estimated LD50 values (measured) and LD50 values greater than the highest tested dose of formulated products plotted against their predicted values (based on active substance content and toxicity) are shown in Figure 1 for all data pairs. The 1:1 line represents equality between the predicted and the measured LD50 values. Values on or below this line indicate where the prediction gave lower (more conservative or more acutely toxic) LD50 values than the measured values. To give an indication of how accurate the predicted LD50 values were when giving rise to a higher (less conservative or less acutely toxic) value, 2-fold, 5-fold, and 10-fold factors are also superimposed above the 1:1 line (dotted and dashed lines). Cases in which the prediction was not close to the measured toxicity were investigated further to identify any limitations to the prediction approach.

The complete dataset (including point-estimated and values greater than the highest dose tested) demonstrates that 79% of predicted LD50 values were lower (more conservative) than the corresponding measured value. Furthermore, 90% of predictions were within 2-fold of the measured value. This increased to 98% and 99% of predictions being within 5- and 10-fold of the measured values, respectively. Less than 1% of predictions (3/383) were more than 10-fold higher (less conservative) than the equivalent measured value.

The dataset was then filtered to determine which data represented the highest quality data in the context of this comparative assessment. These data (59 data pairs; see filled symbols in Figure 1) were considered to be those for which all

active substance and formulated product LD50 values were point estimates (where toxicity was measured)—that is, for which no LD50 values greater than the highest dose tested were obtained. When these data alone are considered, 81%, 98%, and 100% of predictions were within 2-fold, 5-fold, and 10-fold, respectively, of the measured values, indicating that predictions made using unbounded data are representative of those generated using point estimated data. No predicted LD50 values were greater than a factor of 10 from the measured LD50 values when only point estimated data were considered.

#### *Impact on vertebrate use*

On average, the numbers of birds used per study for US EPA OCSPP 850.2100 or equivalent and other test guidelines were 43.7 and 38.3, respectively. On average, studies employing OECD test guideline 223 used only 14.3 birds per study, approximately a 65% reduction in bird usage per study. If this reduction is considered across the whole dataset collated in the present study, the significance of this reduction is clear. In total, 13 939 birds were used to generate the formulated product LD50 endpoints. Based on the mean numbers of birds used per study for OECD test guideline 223, if the 77% of studies conducted under US EPA OCSPP 850.2100 or equivalent and the other test guidelines had been conducted according to OECD test guideline 223, the number of birds used to produce the same data would have been only 5480, a reduction of 8459 birds or 61%.

### DISCUSSION

Avian acute toxicity tests with active substances are a key component of environmental safety evaluation; in the present study, however, we question the need for additional testing of the formulated product. We believe these tests add no additional information to that which is already available from the active substance data.

The analysis reported in the present study demonstrates that 97% of formulated products containing only functionally nontoxic active substances (those with an LD50 value greater than the highest dose tested) also give rise to functionally nontoxic formulated products. Only 3% of formulated products analyzed were found to result in an LD50 < 2000 mg formulated product/kg body weight, even though they were formulated with active substances considered to be functionally nontoxic. All these formulated products, despite showing unexpected toxicity, were still found to have relatively low toxicity, with LD50 values ranging from 1139 mg formulated product/kg body weight to 1919 mg formulated product/kg body weight, within 2-fold interlaboratory variability (see below). Formulated product LD50 values are used for either hazard classification or risk assessment. If the hazard classification system in Brazil is considered as an example, formulated products with LD50 values of at least 500 mg/kg body weight but less than 2000 mg/kg body weight are classified as moderately toxic. Therefore, this 3% of compounds that show unexpected LD50 values below 2000 mg formulated product/kg body weight would be classified as moderately toxic, only 1 category above the lowest possible classification (slightly toxic; >2000 mg/kg body wt). Thus, generating this data would not significantly change the classification assumed from using a predicted toxicity. For the remainder of the formulated products assessed in the present study (97%), there would be no change to the classification. If the use in risk assessment is considered, the most toxic of these formulated products (1139 mg formulated product/kg body wt)

would have to be applied at an unusually high rate of approximately 2 kg formulated product/ha [18] to pose an acute risk to birds in a cereal crop in a worst-case first-tier European Union risk assessment scheme [8]. These data clearly demonstrate that functionally nontoxic active substances, when formulated, remain functionally nontoxic; and even the minimal numbers of exceptions identified are highly unlikely to demonstrate a significant increase in toxicity.

When active substances show measurable toxicity to birds (i.e., have point estimated LD50 values), we have demonstrated that formulated product toxicity can be predicted accurately using simple additivity assumptions. Based on the LD50 of active substances, LD50 values for formulated products were predicted accurately such that 100%, 98%, and 81% were within 10-fold, 5-fold, and 2-fold, respectively, of the endpoints derived from actual studies. The adaptation of Finney's estimation method used in the present study is sufficiently robust that even when the toxicities of the active substances are not fully characterized (unbounded LD50 values), the prediction for the formulated product is still relatively accurate. In the validation tests for OECD test guideline 223 [16], 2 substances were tested (methyl chlorophenoxyacetic acid and isazofos) among 4 laboratories. The differences between LD50 values generated in each laboratory were between 1.7-fold and 2.0-fold of each other, suggesting that a 2-fold difference represents a reasonable estimate of interlaboratory variability. In addition to this interlaboratory variability, there is likely to be a degree of intralaboratory variability (i.e., variability between repeat experiments in the same laboratory) and interspecies variability (between the species tested with the active substance and that for the formulated product). Therefore, for 90% of all the predictions in the present analyses to be within this range demonstrates remarkable accuracy. Furthermore, the validation tests also reported that the variance of LD50 estimates calculated for repeat study data generated with US EPA FIFRA 71-1 single-stage, 60-bird design was 4.52 times greater than the variance of LD50 estimates calculated for OECD test guideline 223. Because most of the data included in this database (77%) were generated according to a guideline related to US EPA FIFRA 71-1 (or a similar single-stage dose-response study design), were conducted at different laboratories, and contain variability associated with species differences between active substances and formulated products, it could be reasonable to conclude that variation nearer to 5-fold is a normal level of variability for these types of tests. Therefore, the toxicity of 98% of the formulated products included in this analysis was reliably predicted within a factor of 5.

Ten formulated products (out of 383) were found to have predicted toxicities less than 5-fold that of their respective measured values. Less information is available to indicate the toxicity of the coformulants (e.g., solvents, dispersants) to avian species compared with mammals. Therefore, when available, the toxicity of the coformulants was investigated for birds or mammals. There were no clear instances in which the major coformulants appeared to have significant avian or mammalian toxicity.

All but 1 of the formulated products showing greater than 5-fold difference between measured and predicted LD50 values were found to contain at least 1 LD50 value greater than the highest tested dose (measured formulated product or active substance LD50). In addition, the single formulated product that did contain only point estimated data was found to have a formulated product LD50 of 2293 mg formulated product/kg body weight and therefore is above the recognized limit dose for

this test type (2000 mg formulated product/kg body wt) and thus is of little concern to avian species.

Three predicted LD50 values were found to be more than 10-fold higher than the respective measured values. All 3 of these formulated products contained very low levels (<10%) of active substance. This means that the prediction is based on a very small amount of possible toxic components within the formulation, and in all cases the formulated products contained at least 1 active substance with an LD50 value greater than the highest dose tested. In 1 instance the formulated product toxicity test was conducted at a single limit test dose of 2000 mg formulated product/kg body weight, and 60% mortality was observed. The toxicity in this case was not investigated further to generate a full dose response or with any replication, and thus the validity of that study and the LD50 value generated (<2000 mg formulated product/kg body wt) could be questioned. In addition, the same comparison was conducted by using the acute mammalian data (LD50 for the rat), and all 3 formulated products were found to have similar relationships when the prediction was compared with the measured formulated product value. Similar relationships with the same formulated product in both mammal and avian species suggest that there could be an unidentified toxic component within the formulation causing the higher than expected toxicity. However, one must remember this represents only a very small proportion of the large dataset examined in the present study. In regulatory terms, mammalian data would be available to identify the small number of instances in which formulated product toxicity may not be effectively predicted from its constituent active substances. In such cases, an avian toxicity test could be performed to address any uncertainty; as has been shown in the present study, however, this is appropriate in only an extremely limited number of cases.

The dataset used in the present study has presented the unique opportunity to quantify the reduction in animal numbers achievable by using a sequential test design. At the request of the OECD, test guideline 223 was developed to determine whether the number of birds required to establish the acute oral LD50 could be reduced. The sequential test design is statistically designed to estimate the LD50, slope, and confidence limits using the least number of birds. For studies on formulated products in the present dataset, a per-study saving of approximately 65% of birds was estimated. If OECD test guideline 223 had been used to generate all of the formulated product data assessed in the present study, a reduction in the use of nearly 8500 birds would have resulted. Therefore, when avian toxicity information is required for formulated products (e.g., when mammalian data suggests this is appropriate), we would strongly recommend the use of OECD test guideline 223 to generate these data.

### CONCLUSIONS

The present data analysis presents a compelling case for a global step change in avian testing for pesticide regulatory purposes. It is possible and necessary to reduce the number of birds used in the testing of formulated products without compromising the integrity of the toxicology database used to make regulatory decisions. Functionally nontoxic active substances generally remain functionally nontoxic when formulated. Therefore, 97% of formulated product testing often fails to produce any adverse findings at limit doses and thus provides no additional data to a registration. In addition, when active substances that show measurable toxicity are formulated, the toxicity of the product can be reliably predicted by using simple

calculations. The small numbers of exceptions may be expected to be captured by reference to the mammalian acute toxicity data on the active substance(s) and formulation. Therefore, a nontest strategy, as is used in the European Union and North America, could be employed in other regions with no compromise in environmental safety standards. This would result in a significantly reduced number of avian acute oral toxicity tests being conducted for global formulated products. When bird acute toxicity studies are still mandated, it is recommended that OECD test guideline 223 should be employed to further reduce the number of birds used in testing.

**Acknowledgment**—The authors thank the UK National Centre for the Replacement, Refinement, and Reduction of Animals in Research for providing a forum for the initiation of this project, and also M. Douglas (DOW AgroSciences), K.E. Brugger (DuPont), M. Ebeling (Bayer), and N. Kreling (BASF) for providing data and constructive edits to the manuscripts. The authors also thank 3 anonymous reviewers for valuable contributions to the manuscript.

### REFERENCES

1. Chinese Ministry of Agriculture. 2007. Data requirement of pesticide registration (DRPR)—China. Ministry of Agriculture Decree 10. Beijing, China.
2. Indian Ministry of Agriculture. 1968. *The Insecticides Act 1968—India*. 46 Act of 1968. New Delhi, India.
3. Brazilian Institute of Environment and Renewable Natural Resources. 2009. Manual application for environmental assessment: Pesticides and related. DIQUA CG ASQ. Brasilia, Brazil.
4. Brazilian Institute of Environment and Renewable Natural Resources. 1996. *Regulation 84—Brazil*. Brasilia, Brazil.
5. US Environmental Protection Agency. 2007. Pesticides: Data requirements for conventional chemicals. 40 CFR Part 158. *Fed Reg* 72:60933–60988.
6. European Commission. 2009. Regulation (EC) 1107/2009 of the European Parliament concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. *Official J Eur Union* L309:1–50.
7. Scholz S, Sela E, Blaha L, Braunbeck T, Galay-Burgos M, García-Franco M, Guinea J, Klüver N, Schirmer K, Tanneberger K, Tobor-Kaplon M, Witters H, Belanger S, Benfenati E, Creton S, Cronin M, Eggen R, Embry M, Ekman D, Gourmelon A, Halder M, Hardy B, Hartung T, Hubesch B, Jungmann D, Lampi M, Lee L, Léonard M, Küster E, Lillicrap A, Luckenbach T, Murk A, Navas J, Peijnenburg W, Repetto G, Salinas E, Schüürmann G, Spielmann H, Tollefsen K, Walter-Rohde S, Whale G, Wheeler J, Winter M. 2013. A European perspective on alternatives to animal testing for environmental hazard identification and risk assessment. *Regul Toxicol Pharmacol* 67:506–530.
8. European Food Safety Authority. 2009. Guidance of EFSA: Risk assessment for birds and mammals. Question EFSA-Q-2009-00223. *EFSA J* 7:1438.
9. Schmuck R, Pflueger W, Grau R, Hollihn U, Fischer R. 1994. Comparison of short-term aquatic toxicity: Formulation vs active ingredients of pesticides. *Arch Environ Contam Toxicol* 26:240–250.
10. Creton S, Douglas M, Wheeler J, Hutchinson T. 2010. Challenging the requirement for chronic fish toxicity studies on formulated plant protection products. *Toxicol Lett* 199:111–114.
11. Creton S, Clook M, Wheeler J. 2013. Application of the threshold approach for acute fish toxicity testing to plant protection products: A proposed framework. *Chemosphere* 95:195–200.
12. US Environmental Protection Agency. 1982. Guideline 71-1: Avian single-dose oral LD50 test. In *Pesticide Assessment Guidelines, Subdivision E—Hazard Evaluation: Wildlife and Aquatic Organisms*. EPA 540/9-82/024. Washington, DC, pp 33–36.
13. Organisation for Economic Co-operation and Development. 1996. Report of the SETAC/OECD workshop on avian toxicity testing. OECD/GD(96)166. Paris, France.
14. US Environmental Protection Agency. 2012. Ecological effects test guidelines OCSPP 850.2100, Avian acute oral toxicity test. Washington, DC.
15. Organisation for Economic Co-operation and Development. 2010. Guidelines for the testing of chemicals, avian acute oral toxicity test. Paris, France.

16. Organisation for Economic Co-operation and Development. 2010. Report of the test method validation of the avian acute oral toxicity test (OECD test guideline 223). *OECD Series on Testing and Assessment 131*. Vol ENV/JM/MONO (2010) 29. Paris, France.
17. Junghans M, Backhaus T, Faust M, Scholze M, Grimme LH. 2006. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. *Aquat Toxicol* 76: 93–110.
18. Garthwaite DG, Hudson S, Barker I, Parrish G, Smith L, Pietravalle S. 2013. Arable crops in the United Kingdom (including aerial applications 2012). Pesticide Usage Survey Report 250. Food and Environment Research Agency, London, UK.