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Concerns of CropLife America Regarding the Application and Use of the U.S. EPA’s Toxicity Reference Database
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In a recent article in EHP, Martin et al. (2009) reported classifying the relative toxicity of chemicals using the U.S. EPA’s (U.S. Environmental Protection Agency) Toxicity Reference Database (ToxRefDB). The authors profiled results from in vivo chronic toxicity/carcinogenicity studies across 310 chemicals currently contained within ToxRefDB. This database has been suggested to be a model for development of predictive signatures of toxicity and for validation of the U.S. EPA’s ToxCast research program (Martin et al. 2009). Although the goals of the U.S. EPA program are worthy of our support because they promote both the prediction of response for chemicals with unknown activity and the avoidance of unnecessary testing in animals, CropLife America has several concerns regarding the application and use of ToxRefDB, as described by Martin et al.

First, we at CropLife America understand that ToxRefDB is not intended for risk assessment purposes. However, in their Table 3, Martin et al. (2009) present a list of 109 chemical compounds according to a “relative potency” grading system (using a scale based on lowest-effect dose levels/end points from chronic toxicity studies) and whether or not tumors occurred according to multigenerations/multispecies/multispecies. Thus, the summary presented in Table 3 represents a hazard ranking system based on relative potency. Such systems are used as tools by some regulatory bodies to make decisions (e.g., the European Union, the State of California under Proposition 65). As a result, it is possible, and even likely, that the data in Table 3 could be used to support regulatory action based solely on this relative potency ranking, which is outside the context of the formal risk assessment process. Even a cursory review of the chemicals listed in Table 3 reveals the presence of many currently registered food-use pesticides that have not been found to pose any unacceptable cancer risk. Therefore, additional care should be taken in the future with regard to any potential rankings using ToxRefDB analyses.

Second, it is problematic that some data entries for chemicals listed in Table 3 (Martin et al. 2009) demonstrate an absence of available information. For example, we checked the accuracy of the Table 3 entry of “N” indicating “not assessed (no study available).” This entry appeared for 12 chemicals (pesticides) that denoted a lack of multispecies carcinogenicity data. In six cases (i.e., pyraclostrobin, dichlorvos, alachlor, captan, maneb, and propargite) the U.S. EPA website of Reregistration Eligibility Decision (RED) documents (U.S. EPA 2009) indicates that, contrary to information presented in Martin et al.’s Table 3, multispecies carcinogenicity studies are available. This inconsistency involving readily available information from RED documents and from data evaluation records (DERs; used primarily by the ToxRefDB) should be addressed to ensure future consistency and completeness of the data. This additional check of existing information by the ToxRefDB is especially important given the statement by Martin et al. (2009) in their abstract that “these data are now accessible and mineable within ToxRefDB and are serving as a primary source of validation for U.S. EPA’s ToxCast research program in predictive toxicology.” Any validation work performed with an incomplete database would be questionable.

Third, we would prefer greater transparency when analyzing “cancer datasets” involving grouping of nonneoplastic proliferative lesions with preneoplastic lesions/neoplastic lesions. Because this type of grouping of nonneoplastic proliferative lesions with neoplasia is not standard evaluation practice (Williams et al. 2008), we at Crop Life America would prefer future ToxRefDB interpretation to identify specific terminology used with regard to scoring system(s) of end point progression schema [such as used by Martin et al. (2009) in their Figure 3A]. Identification of specific terminology and key events for nonneoplastic proliferative lesions and preneoplastic lesions would increase transparency for future publications. Martin et al. stated in their “Results” that they used their method to increase species concordance, which in turn, probably increased the relative power of the statistical analysis. However, it is well known that hyperplastic lesions, for instance, do not always progress to tumors (Klaunig and Kamendulis 2007). By grouping nonneoplastic proliferative lesions with neoplasia, Martin et al. may have increased species concordance and statistical power but may have failed to fully consider the biological plausibility and/or consequences of the grouping. Moreover, the use of this controversial application in the interpretation of the analyses presented by Martin et al. casts some doubt on their validity.

Finally, our last concern involves implementation of results from in vivo testing, which Martin et al. (2009) compared with results of in vitro testing. The authors failed to discuss the issue of chemical activation and detoxification. It is a general principle of toxicology that toxicity of chemicals can be directly dependent on metabolism (Kemper et al. 2008). Metabolic pathways exist in intact animals but not in isolated cells. Therefore, both pharmacokinetics and metabolism are important biological components of the toxicity profile of any chemical. It is not clear that the current analyses using the ToxRefDB (Martin et al. 2009) took this into consideration.

In conclusion, based on our four concerns given above, we hope that the authors will address these points to increase the degree of clarity and consistency of interpretation of analyses using the ToxRefDB.

E. J. is employed by a trade association whose members manufacture and use chemicals in ToxRefDB.

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U.S. EPA’s Toxicity Reference Database: Martin and Dix
Respond
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We appreciate the letter from Janus of CropLife America commenting on that group’s assessment of the database and our article (Martin et al. 2009a) from its perspective as an agriculture and pest-management trade organization. We also appreciate the CropLife America’s continued interest in the
U.S. EPA’s (U.S. Environmental Protection Agency) ToxRefDB and ToxCast research programs, including the review of much of the data entered into ToxRefDB. However, Janus’s comments do not address the ToxRefDB applications presented in our article, but instead create hypothetical uses of the database and reported data.

For example, in Table 3 of our article (Martin et al. 2009a), we presented multi- gender, multisite, and multispecies rodent tumororigens in order to provide data in a systematic and computable format for predictive toxicity models incorporating potency values. In contrast, Janus and CropLife America refer to a hypothetical regulatory application of this same animal tumorigenicity data in a ranking system never suggested in our article.

The U.S. EPA has gone to great lengths to make ToxRefDB and its development as transparent as possible. Three manuscripts and data sets have been published to date (Knudsen et al. 2009; Martin et al. 2009a, 2009b), and the standardized vocabulary and a version of the database are available on the ToxRefDB website (U.S. EPA 2008b). We will continue to make every effort to publicly release information from ToxRefDB as it continues to develop.

We recognize the complexity of pathologic progression to cancer. The end point progression scheme we presented (Martin et al. 2009a) included aggregation of proliferative, preneoplastic, and neoplastic lesions for the development of predictive signatures from in vitro data coming from the ToxCast research program (U.S. EPA 2008a). This approach is not controversial in the context of predictive toxicology research and is supported by the literature (Cohen and Arnold 2008; Hanahan and Weinberg 2000).

We agree that it is important to incorporate pharmacokinetics and metabolism, including chemical detoxification and activation, into predictive toxicology efforts. However, this issue is outside the scope of our article (Martin et al. 2009a) and is being addressed in other aspects of the ToxCast research program.

Two additional papers on multigenerational reproductive and prenatal developmental toxicity studies in ToxRefDB have been recently published (Knudsen et al. 2009; Martin et al. 2009b), again with the primary goal of providing diverse end points for predictive modeling as part of the ToxCast research program (Dix et al. 2007). Of toxicity end points in ToxRefDB, we are only those of sufficient quality for predictive modeling, and we are taking care to distinguish between missing versus negative data.

We view ToxRefDB as a valuable resource to the scientific community and one in which the U.S. EPA, stakeholders, and other interested parties can work together to ensure the success of ToxRefDB and the larger ToxCast effort.

The authors declare they have no competing financial interests.

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This response does not necessarily reflect official U.S. EPA policy.

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Nanotechnology-Related Environment, Health, and Safety Research
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We were very interested to read the article by Schmidt (2009) about the increasing number of nanomaterials and their potential effects. We were especially interested in the similarities between carbon nanotubes and chrysotile asbestos fibers. The widespread use of asbestos-like substances with similar putative carcinogenic potential could result in the development of other unexpected types of cancer.

The potential carcinogenic risk of nanomaterials that are structurally similar to asbestos and have been used in many industrial fields in the last few years has been highlighted by Carter (2008). Some studies conducted in animal models suggest that nanomaterials cause free radical–mediated cellular DNA damage and consequently have an asbestos-like carcinogenic action (Poland et al. 2008).

In addition to mesothelioma (the typical cancer index of exposure) or lung cancer, asbestos can cause other types of cancer. On the basis of our clinical experience, we hypothesize that at least a portion of bile ducts cancers (i.e., cholangiocarcinomas) are caused by exposure to this known carcino- genic agent.

From 2002 to 2008 we treated 258 patients with cholangiocarcinoma at our institute. Over the previous year, we carefully interviewed 66 consecutive patients using a standardized questionnaire asking about their exposure to asbestos and other known risk factors linked to bile duct carcinogenesis (Khan et al. 2008). We collected each patient’s remote, recent, and occupational clinical history. In addition to the association with known risk factors for the onset of cholangiocarcinoma, we assessed occupational or household exposure to asbestos in 24 patients, 10 of whom did not have other certain risk factors (Table 1; Brandi et al. 2008).

Asbestos fibers cause cancer through chronic inflammation, amplifying the production of oxygen radicals, cytokines, growth factors, and proinflammatory factors responsible for both impaired antioxidant and control cell proliferation and apoptosis mechanisms in target cells (Manning et al. 2002). In contrast with findings for pleural mesothelioma, the association between exposure to asbestos and the development of other tumors, such as gastrointestinal and

Table 1. Characteristics of patients.

| Tumor site | No. of patients | Exposure to asbestos | Possible modality of exposure | Certain added risk factors |
|------------|----------------|----------------------|-------------------------------|---------------------------|
|            | Household | Occupational | Ingestion | Inhalation | Absent | Present |
| ICC        | 10       | 7/10       | 4/10     | 7/10       | 4/10 | 6/10 | 4/10 |
| Klatskin tumor | 6       | 3/6        | 3/6      | 3/6        | 3/6 | 3/6 | 3/6 |
| Ampulla of Vater | 1       | 1/1        | –        | 1/1        | –   | 1/1 | 1/1 |
| Main hepatic bile duct | 1       | –          | 1/1      | –          | 1/1 | –   | 1/1 |
| GBC        | 6        | 6/6        | –        | 6/6        | –   | 6/6 | –   |

Abbreviations: GBC, gallbladder carcinoma; HCV, hepatitis C virus; ICC: intrahepatic cholangiocarcinoma. Data updated from Brandi et al. 2008.

aHCV infection or exposure to chemical substances used in industry, sporadic contact with chemical substances used in farming, alcohol-related liver cirrhosis or gallstones, or ulcerative colitis and primary sclerosing cholangitis.
bile duct cancers, has not been univocally demonstrated (Gamble 1994).

The putative increased risk of bile duct cancer in subjects exposed to asbestos may be due to different mechanisms. The asbestos fibers cross the alveolar barrier by inhalation or penetrate the gastrointestinal mucosa by ingestion. They then reach the interstitial environment and circulatory system through lymphatic vessels and are finally delivered to all tissues, namely the liver and bile ducts (Miserocchi et al. 2008), where they may start a malignant transformation process (Wingren 2004). In addition, asbestos fibers may reach the bile ducts through the papilla of Vater from the intestinal lumen by retrograde reflux, as do bacteria, and remain in the gallbladder for a long time.

In the near future we may have to consider asbestos as another factor accounting for the etiopathogenesis of cholangiocarcinomas that may explain the otherwise mysterious increasing incidence of intrahepatic cholangio carcinomas in Western countries.

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In the letter by O’Brien et al. [Environ Health Perspect 117:A385–A386 (2009)], the competing financial interest declaration was incorrect. The correct declaration is as follows:

Karen Peabody O’Brien is executive director of Advancing Green Chemistry, a not-for-profit organization that receives support from several private foundations (listed online at http://www.AdvancingGreenChemistry.org/AdvancingGreenChemistry/About_Us.html) to support efforts to build the field of green chemistry. J.P. Myers is founder, chief executive officer, and chief scientist for Environmental Health Sciences (EHS), a not-for-profit organization that receives support from several private foundations (listed online at http://www.environmentalhealthnews.org/about.html) to support EHS’s mission to advance public understanding of environmental health sciences. John Warner is president of Warner Babcock Institute for Green Chemistry, a private company that applies the principles of green chemistry in the synthesis of new materials and the redesign of chemical processes.

In the letter by Wilson and Schwarzman [Environ Health Perspect 117:A386 (2009)], the last sentence in the first paragraph was incorrect. The corrected sentence is as follows:

We would add that public policy that accurately reflects current science—and the needs of the chemicals market—is instrumental to the widespread adoption of green chemistry.

EHP apologizes for the error.

In the article by La Merrill et al. [Environ Health Perspect 117:1414–1419 (2009)], the keys in Figure 3B and Figure 5C should have been in Figure 3C and Figure 5D, respectively. The corrected figures are provided below.

EHP apologizes for the errors.

Figure 3. Diet and maternal TCDD exposure effects on body composition and fasting blood glucose. (A) HFD increased postnatal D2 body weight (mean ± SE; n = 27–31 at PNDs 0–26 for HFD and n = 28 at PND35 for LFD). (B) HFD (n = 26 mice) increased percent fat at PND35 relative to LFD (mean ± SE; n = 28 mice). (C) Fasting blood glucose was increased by HFD and maternal TCDD-treated (n = 5 litters) compared with HFD and maternal vehicle-treated (n = 6 litters) female progeny at PND35 (mean ± SE). Because diet, but not TCDD, changed body weight and percent body fat, these analyses were done on individual D2 mice, with TCDD- and vehicle-treated D2 mice pooled within diet. *p < 0.05. **p < 0.0001.

Figure 5. Maternal TCDD exposure and effect of diet on gene expression. Normalized message levels are represented as mean ± SE. (C) Induction of Ahr was increased by HFD relative to LFD (n = 11 and 10 litters, respectively). Measurements were pooled across TCDD and DMBA groups. (D) Induction of Cyp1b1 by DMBA was decreased compared with vehicle in HFD-fed but not in LFD-fed D2 mice. LFD groups are vehicle (n = 5 litters) and DMBA (n = 5 litters); HFD groups are vehicle (n = 6 litters) and DMBA (n = 5 litters). *p < 0.05.
In the article by Alyea and Watson [Environ Health Perspect 117:778–783 (2009)], the x-axis labels in Figure 2 were incorrect. The corrected figure appears below.

EHP apologizes for the error.

**Figure 2.** Concentration-dependent dopamine efflux patterns for E₂ and XEs at 9 (A) and 5 min (B), using optimal time points for each compound chosen from the 10⁻¹⁴ to 10⁻⁹ M time course (Figure 1). (A) A 9-min dopamine efflux for E₂, DES, endosulfan, DDE, NP, and BPA at concentrations ranging from 10⁻¹⁴ to 10⁻⁹ M. (B) A 5-min dopamine efflux for E₂, dieldrin, NP, and BPA at concentrations ranging from 10⁻¹⁴ to 10⁻⁹ M. Values are means and SEs; numbers per treatment are as follows: E₂, n = 18; dieldrin, n = 12; DES, n = 18; endosulfan, n = 12; DDE, n = 12; BPA, n = 23; NP, n = 15. Points above the zero point line indicate a positive efflux of dopamine from the cells.

*p < 0.05 compared with control. #p < 0.05 compared with E₂ treatment.