Regulatory Toxicology and Pharmacology 67 (2013) S1–S3

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Editorial

Assessing the mammalian toxicity of high-boiling point petroleum substances

Humans are exposed on an ongoing basis to many and varied complex substances[1] and mixtures. These include industrial or automobile emissions, byproducts from chemical reactions such as arise from cooking or grilling or disinfection of drinking water, remnants or byproducts of pesticides or antibiotics applied to the food chain, or frequently used commercial products such as gasoline and other petroleum-derived substances. The papers in this supplement present multiple aspects of a unified approach to evaluating the toxicological hazards of an important class of complex substances, arising from crude oil refining – high boiling petroleum-derived substances (HBPS) containing a wide variety of polycyclic aromatic compounds (PAC).

The National Research Council (2007), "Toxicity Testing in the 21st Century", Section 2, Limitations of Current Testing Strategies, states "...The current system, which relies primarily on a complex set of whole-animal-based toxicity-testing strategies"...has difficulty in addressing the wide variety of challenges that toxicity testing must meet today.

- Test large numbers of existing chemicals, many of which lack basic toxicity data.
- Test the large number of new chemicals introduced each year.
- Evaluate potential adverse effects with respect to all critical endpoints and life stages.
- Minimize animal use.
- Reduce the cost and time required for chemical safety evaluation.

The proposed vision for risk assessment proposed by the National Research Council committee (Section 3) includes chemical characterization, toxicity testing, dose response and extrapolation modeling, and population-based and human exposure data. In Section 3 on chemical characterization they state "... tools for chemical characterization will include a variety of empirical and computational methods... quantitative structure–activity relationship (QSAR) models that predict biologic activity from molecular structure ..."

Along similar lines to the National Research Council thinking, the U.S. EPA National Center for Computational Toxicology developed the ToxCast research program – (Dix et al., 2007). The objective of the ToxCast research program "... is to develop cost effective innovative approaches to rapidly screen and prioritize many chemicals for further toxicological testing ... ToxCast is building computational models to better predict the toxicity potential of environmental chemicals ... prioritize *in vivo* animal testing of products that have limited toxicity data available..."

The papers in this supplement make an important contribution along directions discussed by the NRC and EPA by developing and applying a series of quantitative models to predict multiple categories of toxicity endpoints for an entire class of chemical substances (HBPS). While not utilizing the specific modeling approaches referred to in the NRC and EPA discussions, these papers demonstrate that the models can be used as a screening tool for untested substances in the class, to predict toxicity endpoints and to prioritize substances for further detailed testing. The models are shown to provide reasonable predictions for new compounds as long as the aromatic hydrocarbon content, represented as the Aromatic Ring Class (ARC) profiles fall within the range of the ARC profiles of the substances that were used to develop the predictive models (i.e., are interpolations within the range). A limitation of this approach is that reasonable predictions can only be made for substances with ARC profiles that fall within the model's domain. Thus a "similar" ARC profile can be operationally defined as one that involves interpolation within the range used to develop the models. The greater the breadth of substances that are used to develop the models, the broader is the class of "similar" ARC profiles.

Rice et al. (2009) provides an overview of issues involved in the determination of similarity of complex mixtures, with particular reference to by-products arising from the disinfection of drinking water. The generic issues raised are also relevant to the papers in this supplement. The U.S. EPA (2000) defines a complex mixture as "A mixture containing so many components that any estimation of its toxicity based on its component toxicities contains too much uncertainty and error to be useful. The chemical composition may vary over time or with different conditions under which the mixture is produced. Risk assessments of complex mixtures are preferably based on toxicity and exposure data on the complete mixture."

Rice et al. discusses EPA's recommendations on risk assessment of complex mixtures. When dose response data toxicity data are available for the whole mixture the EPA (2000) recommends using these data for risk assessments because they are most likely to provide the most realistic risk information. When dose response data are not available for the mixture of concern EPA recommends the use of dose response information for a "similar mixture" or group

[1] In the context of this and other papers in this supplement, a "substance" is an entity defined by a CAS number. Substances may be either simple or complex. The majority of the substances produced by the petroleum industry are substances of complex and variable character and fall within the group of substances designated as Unknown or Variable Composition, Complex Reaction Products and Biological (UVCB) substances.

0273-2300/$ - see front matter
http://dx.doi.org/10.1016/j.yrtph.2013.08.006

of similar mixtures. Therefore the determination of whether a mixture under consideration with no direct toxicity testing information is "sufficiently similar" to other mixtures with toxicity testing results available is of considerable importance to the use of whole mixtures methods. EPA does not suggest methodology for evaluating similarity among complex mixtures. At a minimum, similarities in the main components and proportions among them need to be evaluated.

Regulatory risk assessment approaches that use available information on the mixture of interest are usually more relevant and preferred when compared with those simpler approaches used in the past for commercial products, e.g., diesel oil, insecticide mixtures (U.S. EPA, 2000). The first customized regulatory approach for a complex mixture concerned weathering of Aroclors, i.e., mixtures of polychlorinated biphenyls (PCBs), where compositional change over time was addressed by using risk values for the Aroclors with PCB ratios closest to the environmental mixture (Cogliano 1998). Presently the EPA in its IRIS database has oral reference doses for two whole PCB mixtures (Aroclors 1016 and 1254), so such fine tuning is definitely limited (U.S. EPA, 2013). A different method has been developed for the evaluation of total petroleum hydrocarbons (TPH) at contaminated sites where the TPH is divided into several analytically defined aromatic and aliphatic hydrocarbon fractions and then each fraction is assigned an oral and inhalation toxicity value (MADEP 2001).

The complex and variable nature of the substances addressed in this supplement makes a strong argument for customized risk methods. For such risk assessment approaches to be credible, they must be supported by some understanding of exposure and toxicity, and most importantly for regulations, supported by quantitative measures of dose–response. Statistical approaches to complex mixtures are rarely published. In the text, Statistics in Toxicology (Krewski and Franklin, 1991), only one chapter addressed mixtures and only simple identifiable mixtures. While advances have been made since then in toxicological testing and conceptual modeling of complex mixtures (Dennison et al., 2004; Verhaar et al., 1997; Yang et al., 2004), statistical methods have lagged. Statistical chapters in later texts on mixtures also focus on simple mixtures (Mumtaz 2010) and recent journal articles on complex mixtures mostly address the concept of toxicological similarity (Bull et al., 2009; Feder et al., 2009a,b; Marshall et al., 2013; Rice et al., 2009; Stork et al., 2008).

The specific problem represented by petroleum products is that they are UVCB substances. Petroleum substances are comprised of five basic types of molecules, normal paraffins, iso(branched)paraffins, cycloparaffins (naphthenes), olefins, and aromatics, but with increasing molecular weight the numbers of possible isomeric forms become so great that analytical resolution is difficult if not impossible in most cases. The variability results in part because of differences in crude oil composition and also because of differing process conditions. It is also important to note that the substances are manufactured to meet technical requirements which constrain composition to some extent but are compatible with considerable variability.

The papers in this supplement discuss, for a specific group of petroleum-derived substances – high boiling petroleum substances (HBPS) containing polycyclic aromatic compounds (PAC) – an extension of the general "sufficient similarity" approach suggested by EPA for dealing with risk assessment of complex mixtures. As stated in the introduction of Roth et al. (2013) "...a single HBPS is typically composed of at least thousands of chemical compounds and the composition varies...it is not feasible either to test each individual component of a petroleum stream (or even to characterize each individual component – PF) or to test all possible petroleum substances..." Even nominally the same refined petroleum product produced by the same refinery can vary in composition over time because of changes in the crude oil used, refining conditions and product specifications.

The papers in this supplement overcome this problem by representing the chemical composition of the whole mixture by the weight percent profile of each of the 1 through 7 aromatic ring classes (ARC), i.e., the "ARC profile", which can be characterized analytically. The ARC groups are associated with fuels and petroleum products and have successively higher boiling points, varying by over a factor of two across the classes.

Characterizing the HBPS compositions by their ARC profiles permits pooling data across a large number of petroleum substances to develop predictive models for multiple toxic effects – general, developmental, reproductive, and genetic toxicity. In fact the greater the variation in ARC profiles among the petroleum substances in the model development set (within limits), the more widely ranging will be the model's ability to predict the toxicities of new petroleum substances on which direct toxicity tests have not been carried out. The papers following in this supplement discuss the predictions of the various categories of toxicities in detail. While the modeling efforts involve different categories of endpoints and similar but somewhat differing structures they all have the common thread that they combine the ARC profile with dose level, biological characteristics of the test animals, and test conditions to predict toxicological dose response trends or mutagenic potential.

The results of these modeling efforts permit hazard characterization to be inferred for a whole class of petroleum substances even though formal toxicity tests have been carried out on just a relatively small subset of substances. EPA has developed a "benchmark dose" estimation process and computer modeling system – (Gift et al., 2011) and applies it to the estimation of deminimus or safe doses. Based on dose response relations developed from the results of individual toxicity tests on a specific endpoint, e.g., change in liver weight, for an individual substance, the EPA suggests calculating a "benchmark dose" associated with a specific level of toxic effect. This is denoted as BMD_x and represents the dose at which the effect increases or decreases (as physically appropriate) by x percent relative to the control group response. BMD_{10} is often used. The papers in this supplement generalize the notion of a BMD_x to a "predicted dose response", PDR_x. The PDR_x like the BMD_x represents a dose associated with an x percent change in response from the control group response. However it is based on the results of the fitted predictive model which is developed from a subclass of substances for which toxicity tests have been carried out and is applied to estimate risks associated with the entire class of substances, most of which have no directly associated toxicity test results. Consider a particular HBPS within the class and its associated ARC profile. Substituting the profile % weights into the prediction models results in a model based predicted dose response trend like that obtained for individual tested substances. The predicted trends can be used to determine the doses associated with that profile, PDR_x, with estimated x% changes in response from the estimated control (i.e., 0 dose) response. PDR_x can be used as a "point of departure" in direct analogy with the BMD_x suggested by EPA (Gift et al., 2011).

The papers in this supplement on statistical methods for predicting toxicity of complex mixtures are timely and appropriate. The first paper (Gray et al., 2013) provides background for, and summary and discussion of the remaining papers in the supplement. Nicolich et al. (2013) discusses in detail the development, testing, and "corroboration" of the models for repeat-dose toxicity and developmental endpoints. Murray et al. (2013)a,b and Roth et al. (2013) discuss the details of the development and application of models for developmental toxicity and repeat-dose toxicity endpoints respectively. They explain the reasoning underlying modeling decisions such as selection of endpoints, selection of

datasets, and the application of the models to characterize toxicity. McKee et al. (2013) discusses the development of mutagenicity models related to the Ames test. Unlike the other models which have quantitative endpoints the mutagenicity model is a cascading series of binary endpoint models that place the HBPS into one of several Modified Ames score categories, and ultimately as >1 or $\leqslant$1. This describes the propensity toward mutations. Murray et al. (2013)a,b discusses an approach used to estimate the potential for a HBPS to have reproductive effects. Finally the paper by Patterson et al. (2013) discusses a peer review process that was used to evaluate the scientific validity of the papers and to make comments and recommendations.

Collectively the papers in this supplement document a substantial effort to develop a series of statistical screening models that can predict multiple categories of toxic endpoints for an entire class of petroleum HBPS based on their ARC compositional profiles. While the class of models and the class of applicable chemical substances is somewhat specialized, this contribution is in direct line with the thinking and suggestions of national scientific and regulatory authorities such as the National Research Council and the U.S. EPA about the directions in which toxicological research and testing should advance in the 21st century.

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Available online 15 August 2013