APPENDIX A

COMMON MECHANISM GROUPS, CUMULATIVE ASSESSMENT GROUP AND METHODS FOR CUMULATING TOXICITY

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Compounds acting at the same molecular target belong to the same common mechanism group (CMG) and may be identified as such for toxicological purposes. Examples of CMGs include organophosphate pesticides known to act on the enzyme acetylcholinesterase and dioxins, which bind the aryl hydrocarbon receptor. A substantial amount of information is required to determine membership of a CMG, leading to a relatively small number of established CMGs for the purposes of cumulative risk assessment. Based on US EPA methods, preliminary identification of a CMG should be based on one or more of the following criteria: similarities in chemical structures, mechanism of pesticidal action, general mode/mechanism of mammalian toxicity, common specific toxic effect(s). In the absence of detailed mechanistic information or establishment of a common mode of action, it is commonly possible to group compounds for a combined assessment on the basis of less refined criteria such as target organ toxicity. Relative broad assumptions can be used to identify members of a "lower-tier" cumulative assessment group; for example, a common target organ may be sufficient to suggest that dose addition is likely for a given group of chemicals.[1]

EFSA recently developed criteria for inclusion of compounds in a cumulative assessment group (CAG).[2] The general EFSA methodology for classifying pesticides into CAGs is based on identifying compounds that exhibit similar toxicological properties in a specific organ or system. EFSA's Panel on Plant Protection Products and their Residues has taken the first step of applying this methodology to define groups of pesticides exhibiting toxicity to the thyroid and central nervous systems. In addition, the PPR Panel has carried out a significant amount of preliminary work for the development of groups for effects on other organs/organ systems, such as the reproductive system, liver, eye and adrenals. Future work in this area will involve the gradual implementation of cumulative risk assessment for pesticide CAGs.

A variety of methods have been developed for assessing the combined hazard or risk of compounds that affect the same target organ or have a similar mode of action. Cumulative risk assessments generally address multiple stressors and include consideration of how stressors act together rather than individually. The following sections provide an overview of the predominant approaches used to cumulate the toxicity of compounds in the same CAG. In addition, these summaries indicate whether a common mode of action (i.e., CMG) is recommended for use and review published applications of these methods for regulatory purposes. These sections should not be construed as a comprehensive overview of the available methodology; details can be found at the reference cited herein. In addition to cumulating chemical toxicity for mixtures, the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) has developed guidance for evaluating differential impacts of exposure to pollutants based on race, socioeconomic status, age, health status, proximity to point source and other key factors.[3] Since this resource is not designed to assess molecular-level interactive effects, it is not discussed further in the current document.

HAZARD INDEX AND RELATED METHODS

The hazard index (HI) is based on the simple ratio between the exposure level and reference toxicity value, such as an acute or chronic reference dose (RfD), for each chemical in a CAG (equation 1). The series of ratios, known as hazard quotients (HQs) are summed to determine the cumulative HI for the group.

$$HI = \frac{Exp1}{RV1} + \frac{EXP2}{RV2} + \frac{EXP3}{RV3} + \cdots$$

It is not a requirement that the CAG represent at CMG in order to apply the HI method. However, a higher tier assessment is necessary in order to determine a surrogate reference toxicity value for the common toxic effect when this value is not readily available for a given member of the CAG from the toxicology database for that chemical. The combined risk from exposure to compounds in the CAG is considered acceptable when the HI is less than one.

Variations of the HI also exist for expressing the cumulative toxicity of a given CAG. The cumulative risk index (CRI) is calculated as the sum of reciprocal HQs for chemicals comprising the group, and is therefore mathematically the reciprocal of the HI. Alternatively, the reference point index (RPI) uses the uncorrected point of departure, such as a BMD10 or NOAEL, rather than the reference toxicity value and applies an uncertainty factor (usually 100). The RPI method is mathematically identical to calculating an HI; however, the quantitative toxicological endpoint used may correspond to any common toxic effect, rather than the most hazardous acute or chronic toxic effect for which toxicological reference values (i.e., RfDs) are determined. Similar to the CRI / HI relationship, the margin of exposure (MOE) is mathematically the reciprocal of the RPI value. For additional details regarding the mathematical relationships and applicability of these methods, see the article by Boobis et al (2008),[1] the US EPA Framework for Cumulative Risk Assessment,[4] and the ATSDR guidance manual.[5]

TARGET-ORGAN TOXICITY DOSE MODIFICATION TO THE HAZARD INDEX

The target-organ toxicity dose (TTD) method is a refinement of the HI method, developed in order to accommodate the assessment of mixtures of chemical components that do not share the same critical toxic effect.[5] The TTD for each endpoint of concern is calculated using appropriate minimum risk levels (MRLs) and reasonable exposure levels.

As shown in equation 2, the MRL is used for chemicals when the adverse effect under evaluation and the critical effect are the same. The TTD is used for any chemicals in the assessment group that cause a critical effect different from the adverse effect (E) being evaluated. For example, equation 2 represents the HI adjustment for three chemicals in a CAG, where chemical 3 has a critical effect other than liver toxicity.

$$HI_{HEPATIC} = (E_1/MRL_1) + (E_2/MRL_2) + (E_3/TTD_3) \qquad (2)$$

where:

MRL = minimum risk level

TTD = Target-organ Toxicity Dose

$HI_{HEPATIC}$ = Hazard index for liver toxicity

WEIGHT-OF EVIDENCE MODIFICATION TO THE HAZARD INDEX

A weight-of-evidence (WOE) method proposed by Mumtaz and Durkin in 1992 was the first systematic attempt to address the need for information on interactions among components of the mixture.[5] The method incorporates an uncertainty factor (UF) to modify the HI to account for synergistic, potentiating or antagonistic interactions among a pair of mixture components. Previous experiences using the algorithm for specific mixtures to generate the interaction-adjusted Hazard Index have revealed that the method does not generally handle changes in proportions of mixture components.[5] Therefore, this method is best used for predicting whether hazard may be greater or less than indicated by the HI determined assuming additivity. In one example, ATSDR scientists compared the results of the estimated toxicity using WOE with experimentally determined toxicity of the mixture of similarly acting nephrotoxicants (halogenated aliphatics), and found that the WOE approach correctly adjusted for the interactions observed in experimental animal studies.[6] Results show that the WOE evaluations should be target-organ specific.

Operationally, the method requires evaluation of data relevant to joint action for each possible pair of chemicals in the mixture for the purpose of making binary weight-of-evidence (BINWOE) determinations for the effect of each chemical on the toxicity of every other chemical in the mixture. The BINWOE indicates the expected direction of an interaction (i.e., greater than additive, less than additive, additive, or indeterminate). It also scores the data qualitatively using an alphanumeric scheme that takes into account mechanistic understanding, toxicological significance, and relevance of the exposure duration, sequence, bioassay (i.e., *in vivo* versus *in vitro*), and route of exposure. The alphanumeric terms in the scheme can be converted to a single numeric score (WOE) through multiplication of the direction factor by the data quality weighting factor (equation 3). For quantitative analyses, the composite score for interactions (WOE_N) can be used to modify the uncertainty factor and convert the additivity-based HI to an interactions HI.[5]

$$HI_I = HI_{add} \times UF_I^{WOE}$$

where:

HI_I = Interactions-adjusted hazard index

HI_{add} = Hazard index based on additivity

UF_I = interactions uncertainty factor (e.g., 10)

WOE_n = composite score for interactions

RELATIVE POTENCY FACTOR METHOD

For assessment groups in which there is an established common mode of action (i.e., CMG), each chemical exposure can be converted into the equivalent exposure level of one of the chemicals in the group, typically referred to as the index chemical.[4] US EPA originally applied this technique to assessing the cumulative toxicity of aryl hydrocarbon receptor agonists (e.g., dioxins), and termed it the toxic equivalency factor (TEF) method. The potency equivalency factor (PEF) or relative potency factor (RPF) method is considered more generalized, and has been used for compounds such as polycyclic aromatic

hydrocarbons and pesticides such as organophosphates.[1] In using these methods, the potencies of all chemicals in the common assessment group are normalized to a single potency scale relative to the index chemical, as shown below in equation 4. For example, if the chronic NOAEL for a compound (Chemical X) is 10-fold greater than that of the index compound (IC), the relative potency factor for the compound would be 0.1 and exposures to the compound would be corrected by a factor of 0.1 to determine index chemical equivalents. In effect, 10 times more of the chemical would be required to produce the same effect as the index compound.

$$RPF_X = \frac{Potency_X}{Potency_{IC}}$$

Potency is inversely proportional to dose, which leads to derivation of equation 5:

$$RPF_X = \frac{NOAEL_{IC}}{NOAEL_X}$$

Once RPFs have been determined for individual chemicals, the activity of the cumulative assessment group is determined as a sum of the index chemical equivalent doses to provide a cumulative exposure as index chemical equivalents. This total exposure can be compared to the reference toxicity value (e.g., acute or chronic RfD) of the index chemical. The US EPA Office of Pesticide Programs applied the RPF approach to their assessment to the cumulative risk assessments of two distinct classes of insecticides, the organophosphates and *N*-methyl carbamates.

Pathway-specific cumulative exposure can be determined by summing the exposure levels for various members of the cumulative assessment group corrected by their corresponding RPF values, as depicted in equation 6:

$$Cumulative\ Exposure = \sum (Exposure_X \times RPF_X)$$

Organophosphate Insecticides.[7, 8] The organophosphates (OPs) share the ability to bind to and phosphorylate the enzyme acetylcholinesterase in both the central (brain) and peripheral nervous systems. When acetylcholinesterase is inhibited, acetylcholine accumulates and cholinergic toxicity results due to continuous stimulation of cholinergic receptors throughout the entire nervous system. Because of this common mechanism of action, the OPs represent both a Cumulative assessment group (CAG) and common mechanism group (CMG) for the purposes of cumulative risk assessment. US EPA identified food, drinking water and residential/ non-occupational as the three exposure pathways of interest for OPs. The cumulative assessment of potential exposure to OP insecticides in food included OPs that are currently registered in the U.S. or have import tolerances, while the drinking water assessment included OPs used outdoors and can thus reach water bodies. Earlier OP assessments of the residential exposures pathway considered OPs registered for use in the home.

Considering the high quality dose-response data for all routes of exposure, US EPA selected methamidophos as the index chemical for standardizing the toxic potencies, calculating RPFs for more than 30 OP insecticides,[9] and converting exposures of all OPs in the group to methamidophos equivalents. Toxic potencies for the OPs were determined using brain cholinesterase inhibition from female rats measured at ≥ 21 days of exposure. US EPA used an exponential dose-response model to develop benchmark dose estimates at a level estimated to result in 10% female brain cholinesterase inhibition (i.e., BMD_{10}) to determine points of departure for the oral, dermal and inhalation routes. These endpoints were ultimately used to estimate relative potency for the organophosphate CAG. For the exposure assessment, US EPA used the Calendex™ software to integrate various exposure pathways for the OPs and selected

the 21-day rolling average exposure as the best approximation of actual human exposure to match with the steady state brain cholinesterase data. Risk assessors performed a sensitivity analysis for food exposures evaluating the relationship between single day and 21-day rolling average approaches to ensure that risks for the OP group from possible one-time, peak exposures were not underestimated. Based on the simultaneous evaluation of the three exposure pathways (food, drinking water, and residential use) and their associated routes (oral, dermal, inhalation), the margins of exposure (MOEs) at the 99.9th exposure percentile were generally ≥100, the target MOE for reasonable certainty of no harm for the general population.

***N*-methyl Carbamate Insecticides.**[10] The *N*-methyl carbamate insecticides were established as a CMG by US EPA in 2001 based on the shared structural features and their shared ability to inhibit acetycholinesterase by carbamylation of serine hydroxyl group located in the active site of the enzyme. The biological response and toxic effects of acetylcholinesterase inhibition for the carbamates is identical to that discussed above for the OPs. US EPA again identified food, drinking water and residential/non-occupational as the exposure routes of concern; the criteria for including various carbamates in the three exposure assessments was identical to that presented for the OPs. Based on its high quality dose-response data for all routes of exposure, US EPA selected oxamyl as the index chemical for standardizing the toxic potencies and calculating RPFs for each carbamate insecticide. US EPA used the BMD_{10} for brain cholinesterase inhibition to develop points of departure from the oral, dermal and inhalation routes of exposure. The available toxicology data allowed assignment of tailored FQPA safety factors and interspecies uncertainty factors, which were incorporated directly into the respective RPFs. To account for the intra-species extrapolation factor of 10X, the target MOE for the carbamate cumulative risk assessment was 10. The MOEs at the 99.9th exposure percentile were ≥8 for all populations considering simultaneous evaluation of the three exposure pathways and their associated routes using the exposure levels estimated using Calendex™ software.

LABORATORY EVALUATION OF COMPLEX CHEMICAL MIXTURES

Researchers and risk assessors are currently confronted with two dichotomous research tracks to support cumulative risk assessment: one focused on strengthening individual chemical data for use in a Relative Potency Factor (RPF) approach versus prioritizing the development of complex mixture libraries and sufficient similarity assessments. RPF and other component-based approaches are considered "bottom-up" because they are built by incorporating data from individual chemicals into additivity models. Alternatively, experimental evaluation of whole mixtures is "top-down," beginning with the complex mixture and proceeding to a determination of whether the mixture is associated with adverse effects.[11] The National Toxicology Program of the National Institutes of Environmental Health Sciences has engaged in component-based mixture assessments of dose additive interactions as well as the experimental evaluation of complex mixtures. Comparison of these two approaches has shown that risk assessments for some classes of toxic organic compounds, such as the polycyclic aromatic hydrocarbons (PAHs), may not accurately predict the potential for genotoxicity and immunotoxicity. These shortcomings have been attributed to either unidentified active PAHs present in the complex mixture or synergistic potential that produces larger effects than would additive interactions between components.[12] Because data for the complex mixture or a sufficiently similar mixture is rarely available, the Relative Potency Factor (RPF) method of dose additivity may be the rule, not the exception with the current state of the science.

High-throughput screening (HTS) assays are continually being developed to address the resource intensive and time consuming nature of toxicity testing using laboratory animals. The current animal-based toxicity-testing paradigm cannot address the data needs for understanding the potential risks associated with exposure to chemical mixtures, let alone all of the individual chemicals in commerce.[12] Even existing HTS technologies can evaluate hundreds of thousands of chemicals per week per assay. Therefore, applications of the HTS approach to mixtures has promise in resolving the resource and time limitations that limit the fields of mixtures toxicity research and risk assessment. The NTP, US EPA, the National Institutes of Health Chemical Genomics Center, and FDA have established a collaborative research program termed Tox21, which uses robotics technology to screen thousands of chemicals for potential toxicity.[13] In addition, US EPA has initiated the *in vitro* and *in silico* screening of environmental chemicals for targeted testing and prioritization under the ToxCast Program.[14] Between phases I and II of the ToxCast projects, researchers evaluated 976 chemicals across 331 cell-free enzymatic and ligand-binding HTS assays.[15] HTS approaches such as those used in the Tox21 and ToxCast projects could potentially lead to the rapid determination of hazards associated with exposure to individual chemicals and complex mixtures.

MODELING OF INTERACTIVE EFFECTS

As discussed in previous sections, simultaneous or sequential exposure to a mixture of chemicals may cause interactions in the pharmacokinetic (time course for absorption, distribution and metabolism and elimination) and pharmacodynamics (effects on dose/response, target organ toxicity) of the individual chemicals. Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models can be employed to describe the mechanisms of action and tissues responses for individual chemicals and simple (e.g., binary) chemical mixtures.[16] Researchers have developed PBPK/PD models for a number of individual pesticide active ingredients, such as the carbamate insecticide, carbaryl,[17] and the organophosate insecticide, chlorpyrifos.[18] Knowledge of PK or PD interactions can be quantitatively integrated into the PBPK/PD model for the chemical mixture. Although progress has been made in this area, relatively few examples of PBPK/PD models for pesticide mixtures exist in the peer-reviewed literature.

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