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Risk Assessment of Combined Exposures to Multiple Chemicals
Annex 3 to the Workshop Report**

**Series on Testing & Assessment
No. 140**

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OECD Environment, Health and Safety Publications

Series on Testing and Assessment

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**WHO OECD ILSI/HESI International Workshop on
Risk Assessment of Combined Exposures to Multiple
Chemicals: Annex 3**

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FOREWORD

This document is Annex 3 of a report of the WHO OECD ILSI/HESI International Workshop on Risk Assessment of Combined Exposures to Multiple Chemicals which was held on 15-16 February 2011 in Paris, France. The workshop was held following the proposal from the 45th OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology in February 2010.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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WHO case study: N-Methyl Carbamates

Elizabeth Shipp
Anna Lowit
Gary Mihlan
Kevin Crofton

N-Methyl Carbamates case study

- Objective: apply an existing case study to the WHO / IPCS framework for combined exposure assessment
- Selected the cumulative N-methyl carbamates (NMC) risk assessment prepared by the US EPA (2007)
- Group members include aldicarb, carbaryl, carbofuran, methomyl, other related compounds
 - All inhibit AChE in a similar, rapidly reversible manner
- Exposure may occur via dietary (including drinking water), occupational, and / or residential scenarios
 - Some food samples every year in the US contain multiple NMC residues

N-methyl carbamates CRA – Paris / 15 February 2011 – Slide 2 of 9

Tier 0 assessment

- Exposure assessment:
 - Assume exposure to residues of each NMC singly is at 95th or 99th percentile
 - Exposure estimates ranged up to 0.15 mg/kg bw/day
- Hazard assessment:
 - Assume no tox data is available
 - All compounds are in Cramer Class 3, therefore TTC value of 0.0015 mg/kg bw/day
 - Cumulative hazard index of 6.36 (95th percentile) or 10.77 (99th percentile)
- Outcome: margin of safety too small – keep going
- Uncertainties:
 - Is this measure of conservancy warranted?

N-methyl carbamates CRA – Paris / 15 February 2011 – Slide 3 of 9

Tier 1 Assessment

- Exposure assessment:
 - Single-compound exposure assessments suggest that % ARfD exposure is fairly high for some compounds
 - Could envision exposure to high percentage of ARfD for multiple compounds at once
- Hazard assessment:
 - Hazard data actually exists for NMCs
 - Develop relative potency factors based on an index compound
- Outcome: possibility of excess exposure – keep going
- Uncertainties:
 - Would this assessment actually pass at this tier?
Decision to keep going was based on pre-existing evaluation

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Tier 2 Assessment

- Not conducted – the original assessment went straight to probabilistic assessments
- Skipping a tier completely is not the ideal

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Tier 3 Assessment

- Exposure assessment:
 - Probabilistic modeling of exposure using USDA Pesticide Data Program data (residues in commodities) and food intake survey data
- Hazard Assessment:
 - Still using values from Tier 1 hazard assessment
- Outcome: sufficient margin of exposure – STOP
- Uncertainties:
 - Was this high-level assessment necessary, or would the risk assessment have passed at an earlier level?

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 - Jefferson Fowles, Royal Dutch Shell

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Questions

- In this case, was it advantageous to go through Tier 0 despite having a high degree of data?
 - If you have the data, do you have to use all of the data?
- How might the problem formulation determine which Tier is needed?
- Are there key aspects of combined exposure that have been overlooked, omitted, or not realized within the framework as illustrated by this example?

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Questions

- Within the problem formulation:
 - How would the criteria for grouping differ depending on the question that is being asked?
- What are the current barriers / blockers that we face in utilizing this tiered approach?
 - Are there difficult decision points?
- What other guidance would you like to give?
- Any additional questions or discussion points?

Case Study – Carbamates¹

[Text to accompany the Powerpoint presentation of the case study]

Prepared by Elizabeth Shipp, Ph.D (Bayer CropScience on behalf of ECETOC), Anna Lowit, PhD (US EPA), Gary Mihlan, PhD (Bayer CropScience), and Kevin Crofton, PhD (US EPA), 28 January 2011

Introduction

This case study addresses the N-methyl carbamate (NMC) insecticides as an example of a group of substances with a well-understood mode of action and a relatively complete toxicity database. Additionally, they are used on a number of food crops, and the possibility may exist of dietary exposure to more than one NMC insecticide during one day. In combination, the potential for co-exposure, and the high potency of these pesticides, raises concerns about potential health effects from exceeding margins of safety.

Dietary exposure, however, is not the only possible route by which exposure to the NMC insecticides could occur. Exposure may also occur via the drinking water through contamination with NMCs, by the occupational route during pesticide application or re-entry tasks, or by residential exposure following “home and garden” application of NMC insecticides. Due to the high potency of the NMC insecticides, and the possibility of exposure via multiple pathways, the US Environmental Protection Agency (US EPA) has conducted an extensive risk assessment of cumulative exposure to NMC insecticides via multiple routes and pathways (US EPA, 2007e).

This document will examine briefly the toxicity of the N-methyl carbamate insecticides, their uses and potential exposures via the dietary route, and the assessment of the risk of dietary exposure to more than one of these substances. The overall question to be answered by this assessment is: Is there a risk of health effects posed by potential cumulative exposure to multiple NMC insecticides via the dietary route of exposure, given their potency, their use on multiple crops and their detection in multiple agricultural commodities?

Considering a Framework Analysis

The draft WHO/IPCS framework presents a series of questions designed to determine whether or not it is appropriate to group substances in an assessment group for a risk assessment. Those questions will be addressed here for the N-methyl carbamate insecticides.

- What is the nature of exposure, and are the key components known or are there data available on the hazard of the mixture itself?

¹This publication contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

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For the N-methyl carbamates, exposure may take place in the following ways:

- dietary exposure to carbamate insecticides on food or in drinking water; this may be to only one substance, or to multiple carbamates, or
- occupational exposure during application of an insecticidal formulation; this may be on crops or in a residential or other setting, or
- residential exposure through uses on lawns and gardens, or on pets.

The key components of this group are known, although their toxicity has largely been assessed on the basis of single compounds.

- Is exposure likely taking into account the context?

Yes. Quantifiable residues of some of these NMCs have been measured in food monitoring programs in the United States (USDA, 2007). Additionally, these insecticides are relatively stable in water, and thus it can be expected that some of the applied amounts will be present in the drinking water after either agricultural or residential application. As three of the NMCs are registered for residential uses, there is also the potential for residential or non-occupational exposure to NMC residues. However, the potential for exposure to NMCs through either drinking water or residential exposure is not considered in the current document. As described in the US EPA's revised NMC cumulative risk assessment, these two potential pathways of exposure are less significant than exposure through dietary routes and thus they are not included in this evaluation of the framework.

- Is there a likelihood of co-exposure within a relevant timeframe?

Yes. A wide variety of fruit, vegetable, and grain crops are treated each year with NMC insecticides. Given the high use of NMC insecticides, the large number of crops treated, and the frequency with which NMC insecticides are detected in monitoring programs, the possibility of exposure to multiple carbamate insecticides from multiple foods during one given day needs to be investigated.

- What is the rationale for considering compounds in a common assessment group?

The N-methyl carbamate insecticides all act, in both target insects and in mammals, by a rapidly reversible inhibition of the acetylcholinesterase enzyme. This inhibition leads to an accumulation of the neurotransmitter acetylcholine, rather than the usual degradation of acetylcholine shortly after its release into a nerve synapse. Accumulation of acetylcholine then leads to continuous stimulation of cholinergic receptors throughout both the central and peripheral nervous systems, leading to acute cholinergic toxicity.

The NMC insecticides are not the only insecticides which inhibit acetylcholinesterase; rather, they share this same mechanism with the organophosphate insecticides such as chlorpyrifos. However, the NMC insecticides cause rapid onset of effects as well as rapid recovery following inhibition, with maximal inhibition usually occurring between 15 and 45 minutes after exposure and recovery following within a matter of hours at most. The organophosphate insecticides inhibit acetylcholinesterase in a largely non-reversible manner and thus do not share the same time-course of recovery from inhibition. Due to this difference, organophosphate and NMC insecticides have not been considered jointly in one common assessment group, and a common mechanism cumulative risk assessment for the organophosphate insecticides has already been conducted (US EPA, 2002, 2006). The NMC insecticides were established as a common mechanism group by the US EPA in 2001 (US EPA, 2001), and the cumulative assessment prepared for the NMC insecticides then will be used as the basis for this document.

Purpose / Focus of the Assessment

The N-methyl carbamate insecticides considered in this risk assessment have food uses or tolerances in food crops in the United States and thus there may be exposure to multiple NMCs via the dietary route. They have also been assessed by the US EPA in a common-mechanism risk assessment (US EPA, 2007e), and that document is largely the basis for the current evaluation.

The following graphic shows the WHO/IPCS framework for risk assessment of grouped compounds. It is important to note that only as many tiers should normally be worked through as are needed to pass the risk assessment; if crude estimates show that both exposure and hazard are quite low, there is no need to go on to higher tiers and develop more refined data such as Monte Carlo exposure estimates.

Example Tiered Exposure and Hazard Considerations Mixture or Component Based

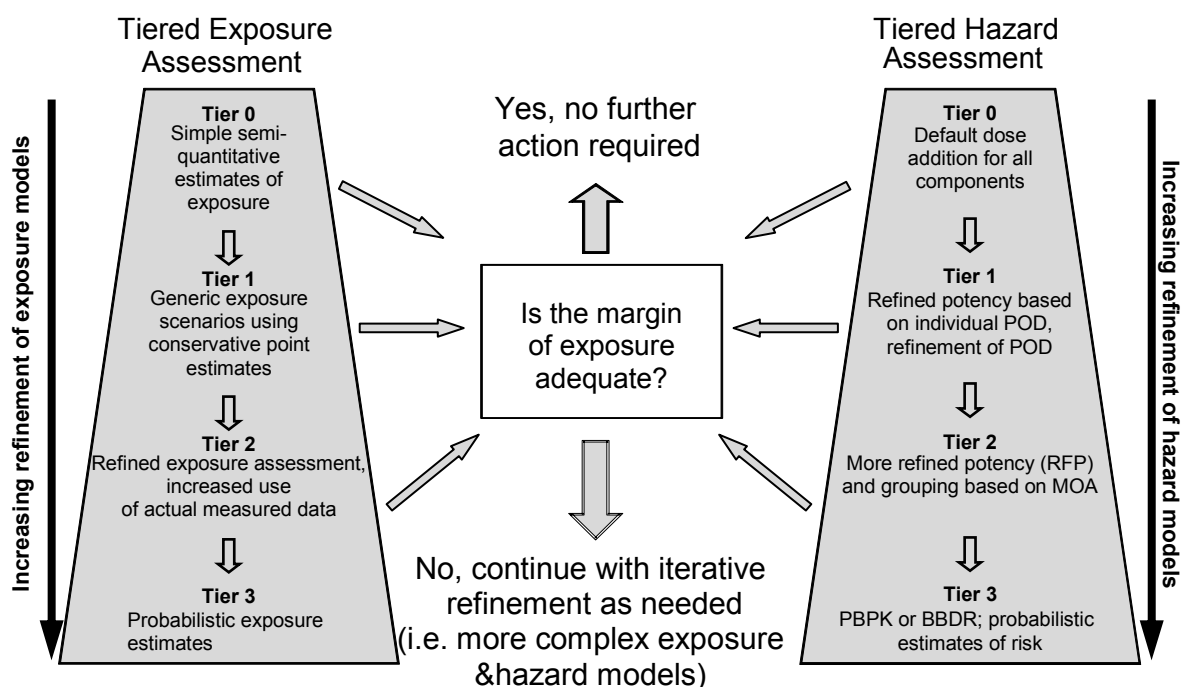


Figure 1. The framework for a common mechanism group risk assessment, as proposed by WHO / IPCS.

The current case study, however, is based on a pre-existing risk assessment for a common mechanism group of substances rather than being an original work. Although not all agricultural or residential pesticides require statistical methods of exposure assessments prior to registration or re-registration, the toxicity of the NMCs and the potential for exposure to residues of multiple NMCs at one time suggested that detailed estimates of exposure should be conducted when these compounds were evaluated as a group. For this reason, the type and amount of data available for this case study especially on the exposure side does not match the tiers outlined in the WHO / IPCS framework. This evaluation is not meant to stand as an independent, original risk assessment of a specific common mechanism group of substances, but as a comparison of the WHO / IPCS framework to an existing assessment in order to see whether the framework is a logical, workable method of analysis.

The Framework Analysis

In the following analysis of potential for cumulative exposure to the NMC insecticides, safety / uncertainty / assessment factors have been used, especially in the hazard assessments at each tier, which differ from those previously proposed by ECETOC. Additionally, the 10% AChE inhibition criterion is used for benchmark dosing (BMD) analysis as used by the US EPA, rather than the 20% criterion commonly used by WHO and other international organizations (International Programme on Chemical Safety. Environmental Health Criteria 104. Principles for the Toxicological Assessment of Pesticide Residues in Food. World Health Organization. Geneva, 1990). The use of these specific uncertainty factors and BMD criteria does not reflect a change in policy by any organization, but a pragmatic use of existing data made available by the US EPA

Similarly, a risk assessment on grouped compounds would only assemble and use that information required to achieve a “passing” risk assessment. In the current case of NMC insecticides, all of the tiers in the WHO framework are examined regardless of whether or not they would have actually been required; this is done in order to provide an example of information which might be used, rather than as an example of a checklist risk assessment.

The compounds to be considered in at least one stage of this evaluation are listed in Table 1 below.

Table 1. Identity and US food-use registration status of N-methyl carbamate insecticides evaluated in this framework analysis

Compound	CAS Number	Registration	Tolerance ¹
Aldicarb	116-06-3	Yes	
Carbaryl	63-25-2	Yes	
Carbofuran	1563-66-2	Revoked in 2009 ²	
Formetanate-HCl	23422-53-9	Yes	
Methiocarb	2032-65-7	No food uses ³	
Methomyl	16572-77-5	Yes	
Oxamyl	23135-22-0	Yes	
Pirimicarb	23103-98-2		Pending
Propoxur	114-26-1		
Thiodicarb	59669-26-0	No food uses ³	

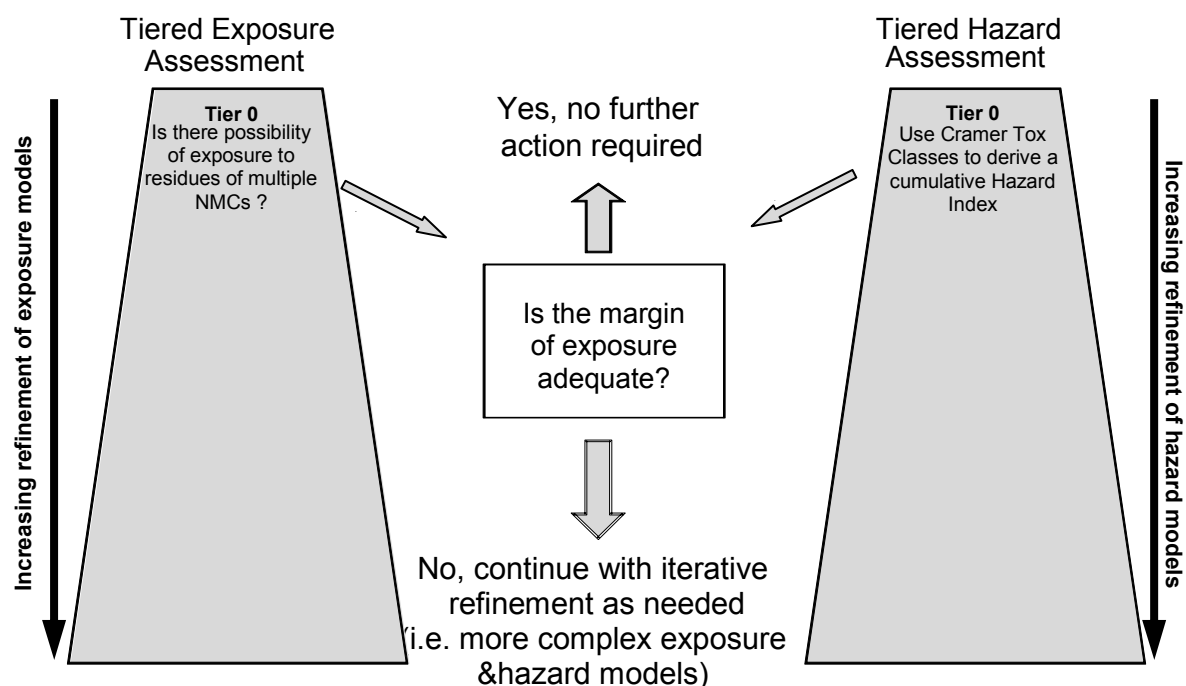
¹ Existence of a tolerance is given only where there is no registered food use.

² Carbofuran was included in the US EPA’s cumulative risk assessment (US EPA, 2007e) and is thus included here, although all uses were cancelled in 2009.

³ As non-food uses of methiocarb and thiodicarb exist and thus exposure may occur, they are included here although dietary residues of these two substances are extremely unlikely.

Tier 0 Exposure and Hazard Assessments

Tier 0 Assessments: Semi-quantitative exposure assessments and default assumption of dose additivity



Tier 0 Exposure Assessment:

The 10 pesticides listed in Table 1 are or have in the past been used either on food crops or in households, and are thereby considered to present potential for exposure. Quantitative exposure estimates for children weighing 10kg (the population group of greatest concern) and consuming 1 kg food using the 95th and 99th percentiles of exposure are shown in Table 2, and were derived from the Dietary Exposure Evaluation Model (DEEM) analysis of dietary consumption based on data from the Continuing Survey of Food Intakes by Individuals (CSFII) from 1996-97 plus 1998 Children's Survey. Food as eaten was converted to amounts of agricultural commodities based on recipe files in DEEM, and agricultural commodity residues were based on the USDA Pesticide Data Program (PDP) monitoring results. Exposure estimates (mg/kg bw/day) were then generated using the DEEM probabilistic assessment of dietary consumption and agricultural commodity residues for the CSFII population. Estimates of exposure were generated for specific age groups using generic exposure factors (EPA. Child-Specific Exposure Factors Handbook, EPA-600-P-00-002B, September 2002, Interim Report). However, only the estimates for the subpopulation group of infants less than 1 year old are shown in Table 2.

Although residue co-occurrence does occur in agricultural commodities, the residues used in the case study assume the 95th or 99th percentile residue level for each individual AI, rather than the co-occurring residues from a sample.

Tier 0 Hazard Assessment:

The Threshold of Toxicological Concern (TTC) concept can be used as a preliminary, rapid screen to determine whether further assessments are needed for a common mechanism group of substances (Boobis et al., 2011). The TTC concept proposes that a de minimis value for toxicity can be identified for many chemicals, such that when structural data are available, SAR can be used to inform TTC (Kroes et al., 2000). The TTC approach identifies an exposure level below which effects are considered unlikely to occur. These exposure levels are based on the physical structure of the chemical in question and on existing toxicity data for a large number of other chemicals previously identified in a database of reference substances for which NOELs are known (Munro et al., 1996). The database employs Cramer classes (Cramer et al., 1978) that classify chemicals into three classes based on potential adverse outcomes. It is widely thought that the Cramer classification, based on the 5th centile NOEL, is a conservative estimate of toxicity that has been used for a variety of assessments (Kroes et al., 2000; Munro, 2008; Drew and Frangos, 2007; Dolan et al., 2005; Renwick, 2005; Smith et al., 2005).

In the Tier 0 Hazard Assessment of this case study, although extensive hazard data exists for each of the NMCs, it is assumed that these data are lacking. A hazard index (the sum of the exposures divided by the reference value for each of the individual compounds of the assessment group) cannot be developed in the absence of hazard data, thus the TTC approach is first used to provide a conservative hazard estimate based on exposure. For each NMC, a Cramer Class was determined using ToxTree (<http://toxtree.sourceforge.net/>). The similarity in structure of the NMCs resulted in assignment for each of the ten substances a Cramer Class 3 value of 0.15 mg/kg bw/day. Incorporation of a standard 100x safety factor for inter- and intra-species uncertainty results in a TTC of 0.0015 mg/kg bw/day for each of the 10 NMCs. The TTC value for each NMC and the estimated daily dose in mg/kg bw/day for each compound can be used to determine a cumulative Hazard Index, as shown in Table 2.

Summary, Tier 0 Exposure and Hazard Assessment

Table 2, below, illustrates the calculation of the Cumulative TTC Hazard Index for 95th and 99th percentile exposure estimates including both food and water exposures if relevant. The dose was calculated based on a 10kg body weight in infants less than 1 year old, the HP-TTC was calculated as the dose divided by the TTC value, and the cumulative HI-TTC was the simple sum of individual-chemical HQ-TTC values. The cumulative Hazard Index clearly exceeds 1 whether the 95th or the 99th percentile value is used, thus further exposure and hazard assessment of the NMC insecticides is required. However, use of the TTC concept provides a conservative and rapid method for evaluating the need for further work.

Table 2. TTC Mixture Model using 95th or 99th Percentile Exposure Data

Component	Cramer Class for Component	TTC, mg/kg bw/day	95 th Percentile			99 th Percentile		
			Estimated Exposure, mg/kg bw/day	Dose, mg/kg bw/day	Hazard Quotient TTC	Estimated Exposure, mg/kg bw/day	Dose, mg/kg bw/day	Hazard Quotient TTC
Aldicarb	3	0.0015	0.00029	0.000029	0.0193	0.000136	0.0000136	0.0091
Carbaryl	3	0.0015	0.000706	0.0000706	0.0471	0.001919	0.0001919	0.1279
Carbofuran	3	0.0015	0.000041	0.0000041	0.0027	0.000091	0.0000091	0.0061
Formetanate HCl	3	0.0015	0.089488	0.0089488	5.9659	0.146534	0.0146534	9.7689
Methiocarb ^a	3	0.0015	0	0	0	0	0	0
Methomyl	3	0.0015	0.000307	0.0000307	0.0205	0.000573	0.0000573	0.0382
Oxamyl	3	0.0015	0.00229	0.0000229	0.1527	0.00839	0.000839	0.5593
Pirimicarb	3	0.0015	0.002215	0.0002215	0.1477	0.003945	0.0003945	0.2630
Propoxur ^a	3	0.0015	0	0	0	0	0	0
Thiodicarb ^a	3	0.0015	0	0	0	0	0	0

^a For methiocarb, propoxur, and thiodicarb, there was no food use or minimal use resulting in no exposure

Cumulative Hazard
Index_{0.95} TTC
6.36

Cumulative Hazard
Index_{0.99} TTC
10.77

Cramer Class: Calculated from ToxTree

TTC: From ToxTree

Exposure: mg/kg bw/day, from food (and water if relevant)

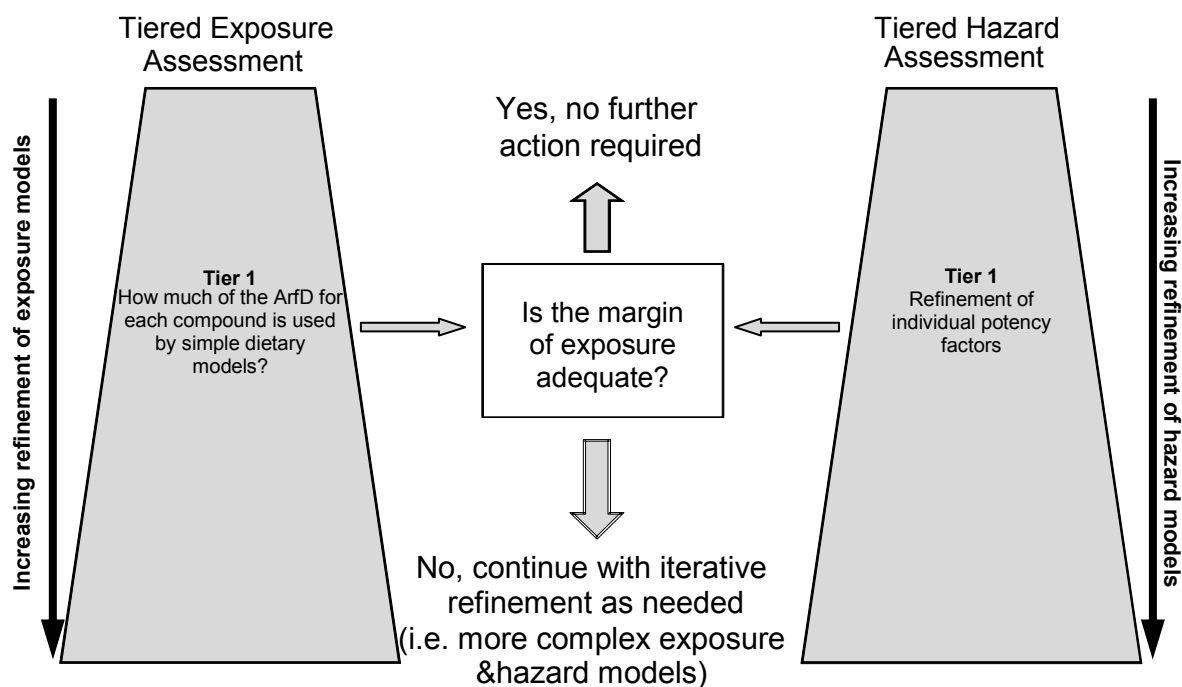
Dose: Assumes a 10 kg body weight (child)

HQ-TTC: Dose / TTC

Cumulative HI-TTC: Sum of individual chemical HQ-TTCs

Tier 1 Exposure and Hazard Assessments

Tier 1. Exposure relative to Acute Reference Doses, and refinement of individual potency factors



Tier 1 Exposure Assessment:

At Tier 1, the exposure assessment is expected to be a generic exposure scenario using conservative point estimates of exposure.

With regard to NMC insecticides, this could include an evaluation of estimated consumption compared to the acute reference dose (ARfD) for each compound. For each compound, the estimated consumption as a percentage of each ARfD is listed in Table 3 as well as the derivation of the reference dose used. The data presented there show that while there is only low estimated exposure to some NMCs (pirimicarb, exposure to 7-10% of the ARfD is expected for all groups examined), some NMCs which are used with greater frequency and on more food crops are estimated to carry a greater likelihood of exposure (carbaryl, with exposure estimated to be 43-68% of the ARfD).

These data were extracted from either the US Reregistration Eligibility Documents or the US Tolerance Petition notices for each compound (US EPA, 1998a, b, 1999, 2007a, b, c, d, e). As maximum residue values were used by the US EPA to calculate predicted exposure, these values are fairly conservative; there is also a great deal of uncertainty regarding the actual incidence of co-occurrence of NMC residues in the diet in any given meal or day. Mining of the USDA PDP data shows that only a few samples tested showed multiple NMC residues each year. However, it is also possible to be exposed to multiple NMC residues through consumption of multiple food items.

The data in Table 3 show that it is possible for a varied diet to contain significant proportions of the acute reference doses of compounds within the same common mechanism group; for example, children 1-6 years of age could consume 19% of the ARfD for aldicarb by eating citrus, sweet potatoes, or potatoes, 81% of the ARfD for oxamyl by eating citrus, sweet potatoes, cucumber, or apple, and 7% of the ARfD for pirimicarb through consumption of asparagus and leafy petiole crops such as celery. The ARfDs for aldicarb and oxamyl are both based on measured inhibition of acetylcholinesterase, while that for pirimicarb is based on clinical signs of neurotoxicity most likely related to acetylcholinesterase inhibition.

Table 3. Acute reference values for NMC insecticides with food-use registrations or MRLs / tolerances, and estimated consumption of those reference values

Compound	Reference Value	Source study	Endpoint	Safety Factors	Age group	% Reference value
Aldicarb	0.001 mg/kg bw/day	Human volunteer	AChE inhibition	100	Children, 1-6 years old	19%
Carbaryl	0.01 mg/kg bw/day	Rat devel. neurotox	FOB changes	100	General population	43%
					Children, 1-2 years old	68%
Formetanate HCl	0.00065 mg/kg bw/day	Comparative AChE study	AChE inhibition	100	Adults, 20-49 years old	16%
					Infants	56%
Methomyl	0.02 mg/kg bw/day	Rabbit teratology	Maternal / fetal tox	300	Infants < 1 year old	27%
					Children 1-6 years old	72%
Oxamyl	0.001 mg/kg bw/day	Rat acute neurotox	AChE inhibition	100	Children, 1-6 years old	81%
Pirimicarb	0.01 mg/kg bw/day	Rat neurotox	Clinical signs	1000	General population	10%
					Children, 1-2 years old	10%
					Children, 1-6 years old	7%
Thiodicarb	0.01 mg/kg bw/day	Rat teratology	Body weight gain	1000	Children, 1-6 years old	31%
					Infants	60%

Tier 1 Hazard Assessment:

In Tier 1, the hazard assessment calls for refining the potency of the individual members of the common mechanism group, based on their individual points of departure, as well as refining those points of departure. In Tier 0, in order to test the TTC methodology, the overly-conservative assumption was made that no hazard data existed for any of the NMCs, while in fact that is not the case.

The NMC insecticides are known to share the same mode of action, namely a rapidly reversible inhibition of the acetylcholinesterase enzyme. A recent paper from the US EPA (Padilla et al., 2007) demonstrated that when administered separately to rats, they share largely similar time courses for reversal of inhibition. The following table summarizes inhibition and reversibility data for the 7 compounds investigated in that paper.

Table 4. Inhibition characteristics of selected NMCs in rat brain and red blood cell after oral administration (Padilla et al., 2007)

Compound	Mg/kg bw	Time to peak effect, h	Maximum % inhibition		Time to 100% recovery, h	
			Brain	Red blood cell	Brain	Red blood cell
Carbaryl	30	0.5	40	10	2	24
Carbofuran	0.5	0.5	40	55	6	6 – 24
Formetanate	10	0.5	85	85	4 – 24	4 – 24
Methiocarb	25	0.5	60	75	4 – 24	4 – 24
Methomyl	3	0.5	53	67	4	4
Oxamyl	1	0.5	50	80	4	4
Propoxur	20	0.25	68	80	4	4

In general terms, it is clear that the NMCs tested here show similar characteristics in their time to peak inhibition of acetylcholinesterase and in the time to recovery of enzyme activity. However, comparison of the doses and their effects show that the potency of the NMC insecticides is not equivalent and that significant brain inhibition (>50%) occurs at relatively low doses for several NMCs. For example, a dose of 1 mg/kg bw produces 50% inhibition of brain cholinesterase at 30 minutes after administration, while a dose of 30 mg/kg bw of carbaryl produces only 40% inhibition of brain cholinesterase at the same 30-minute time point. Clearly, the potency of the different NMCs assessed above (and presumably those examined in the current document which were not studied by Padilla et al) varies with regard to maximum inhibition of acetylcholinesterase.

Although not all of the compounds tested are considered in the risk analysis (due to lack of food uses or tolerances in food items) and not all compounds in the risk analysis were tested, it is safe to assume that this variation in potency extends to those compounds which were not tested by Padilla et al (2007) (aldicarb, pirimicarb, and thiodicarb).

The following hazard assessment data is that presented in the US EPA cumulative assessment of NMC insecticides (US EPA, 2007e). In order to use a pre-existing assessment rather than to derive an original work, the data generated by the US EPA and presented in its cumulative assessment will be used even though it may be more complex than what would necessarily be needed under the WHO / IPCS framework.

In order to derive relative potency factors, the inhibition of brain cholinesterase by each of the NMCs considered in this assessment was determined. Benchmark dose estimation included a meta-analysis of data submitted by the registrants in combination with data generated by the US EPA for each of the substances listed, as well as published or other experimental data. The benchmark dose and ½-life to recovery estimates (not provided here) were derived using a sophisticated dose-time-response

exponential model. The limit of sensitivity for detecting cholinesterase inhibition in either brain is considered to be 10% based on a analysis by US EPA (USEPA, 2002) using data from over 100 studies and over 30 organophosphates. Thus the central estimate of the BMD₁₀ was selected for use in deriving RPFs. The BMD₁₀, BMDL₁₀, and RPF for each of the members of the NMC group are presented below (Table 5). The selection of the limit of sensitivity for deriving a benchmark dose for a given endpoint is a matter of debate, and differs for each endpoint of interest. The use of the BMD₁₀ in this case, rather than the BMD₂₀ commonly used by WHO, ECETOC, and other organizations does not reflect a change of policy by those organizations, but a pragmatic use of the data already generated and analyzed by USEPA in their cumulative risk assessment of the NMCs which forms the basis of the current document.

The modeling summarized here was conducted in order to cover dietary, residential, and occupational uses of the NMCs. As residential and occupational exposures assessed by US EPA's 2007 cumulative risk assessment in addition to exposures through food and water may be through dermal and inhalation routes as well as by the oral route, the index chemical for RPF derivation was selected on the basis of a robust database for acetylcholinesterase inhibition after administration by oral, dermal, and inhalation routes. As the more robust database by all three routes belonged to oxamyl, that substance was selected as the index chemical. The data available for oxamyl included 6 acute oral studies including one study in humans and a comparative cholinesterase study in adult and juvenile (postnatal day 11) rats, and covered a dose range in the rat studies from 0.005 to 15.3 mg/kg body weight, thus providing a broad dose-response range. Although the current document does not cover non-dietary exposures, oxamyl will be retained as the index chemical for this case study.

For three of the compounds considered in this risk assessment, valid and ethically conducted human studies on acetylcholinesterase inhibition were used to adjust the inter-species assessment factor; in the absence of acceptable human data, this value remained at the default value of 10 for others. Where data existed on the relative susceptibility of juvenile and adult experimental animals, these data were also used in deriving a relative potency factor for children. In the cases where no comparison between juveniles and adults was available, a standard 10-fold safety factor ("FQPA safety factor") was applied for children. This additional safety factor is a statutory requirement in the United States and differs from current standard requirements in other markets and from other regulatory agencies. The adjusted inter-species and FQPA safety factors, as well as the resulting RPFs of each compound for children and adults, are shown in Table 6.

Finally, the assumption called for in the preliminary (Tier 0) hazard assessment was that exposure to more than one NMC insecticide would produce an additive response in inhibition of acetylcholinesterase activity. As the potency of the various NMC insecticides tested was apparently different, this assumption was tested by conducting a mixture study in which the proportion of each compound within the mixture was based on its calculated potency (US EPA, 2007a). A dose-response curve was generated using overall doses of the mixture which were predicted to yield between 5 and 60% inhibition of brain and red blood cell acetylcholinesterase activity. The results obtained indicated the predicted additivity, and the inhibition at each dose was very similar to the predicted inhibition of acetylcholinesterase activity (data not shown). This work confirms simple additivity of effects after simultaneous exposure to more than one NMC, despite their differences in potency.

The adjusted relative potency factors for each of the NMCs in this analysis were then used in a refinement of the initial exposure assessment (Table 6).

Commonly, animal-to-human risk assessment for comparison of exposure to a standard reference value uses inter- and intra-species safety factors of 10, or alternatively a margin of exposure between animals and humans of 100. As the inter-species factor is already included in the relative potency factor for each NMC, only the intra-species factor remains. Thus, in the exposure assessment a margin of exposure of 10 (representing the intra-species safety factor) or better indicates acceptable risk.

As noted earlier, the use of these safety factors represents current US EPA practice and does not indicate changes in proposals previously made by ECETOC, WHO, or other organizations.

Table 5. Benchmark doses and relative potency factors for the NMC insecticides examined in this cumulative risk assessment.

Compound	Brain		RPF
	BMD ₁₀ , mg/kg bw	BMDL ₁₀ , mg/kg bw	
Aldicarb ¹	Female = 0.05 Male = 0.06	Female = 0.03 Male = 0.03	4
Carbaryl	Registrant female = 1.60 Registrant male = 1.21 NHEERL male = 5.46 Combined male = 1.58 Moser = 2.63	Registrant female = 1.35 Registrant male = 0.99 NHEERL male = 4.15 Combined male = 1.11 Moser = 2.03	0.15
Formetanate HCl	0.11	0.06	2.18
Methiocarb	1.31	0.56	0.18
Methomyl	0.36	0.2677	0.67
Oxamyl	0.24	0.18	1.00
Pirimicarb	11.96	6.98	0.02
Thiodicarb	0.27	0.23	0.89

¹ The sulfone and sulfoxide metabolites of aldicarb are not listed in the current risk assessment although they are represented in the higher-tier exposure assessments.

Table 6. Relative potency factors, inter-species and FQPA safety factors, and adjusted relative potency factors for children and adults for each of the NMC insecticides of interest.

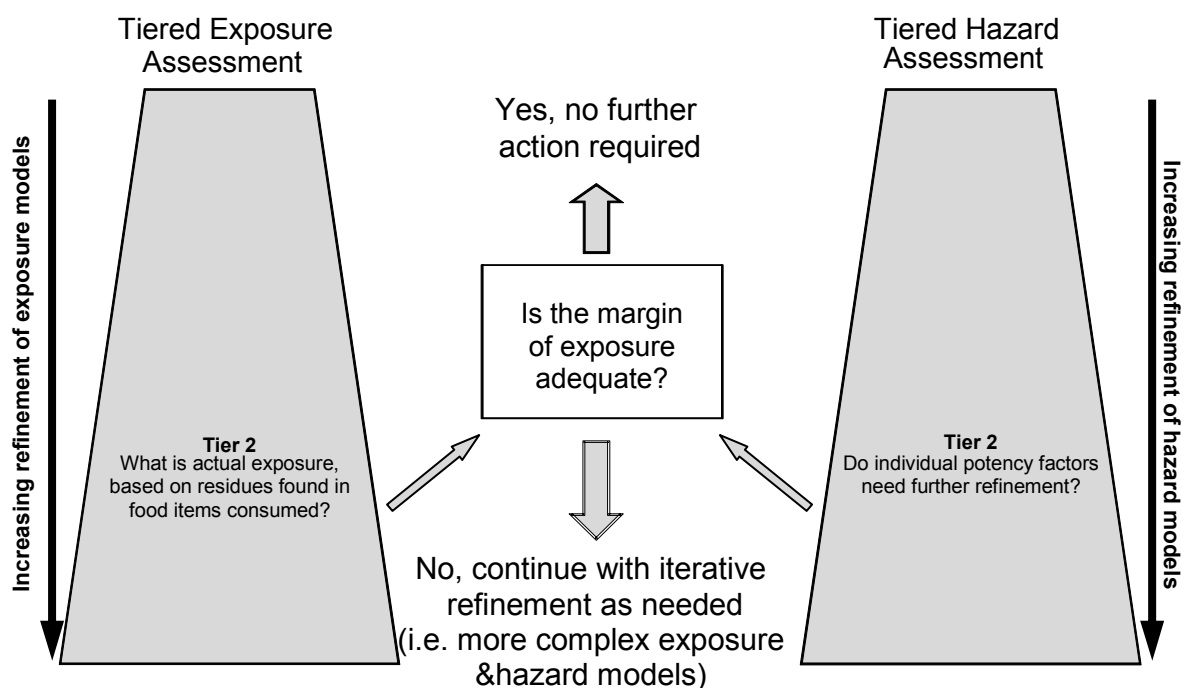
Compound	Oral RPF	Inter-species SF	FQPA SF	Adjusted RPF, children	Adjusted RPF, adults
Aldicarb	4	2	2	16	8
Carbaryl	0.15	10	1.8	2.7	1.5
Formetanate HCl	2.18	10	2.03	44	22
Methiocarb	0.18	10	10	18	1.8
Methomyl	0.67	5	3.05	10	3.3
Oxamyl	1	3	3.48	10	3
Pirimicarb	0.02	10	10	2	0.2
Thiodicarb	0.89	10	10	89	8.9

Summary, Tier 1 Exposure and Hazard Assessment

Simple additivity of the effects of NMC insecticides was confirmed through experimental investigation, and refined potency factors for each of the compounds of interest were developed. However, as there is the possibility for significant exposure to residues of multiple NMCs in one day, even if consumption of each individual compound is below the reference dose, the Tier 1 exposure assessment does not provide sufficient margin of exposure to end the evaluation at that Tier. Further assessment of exposure is needed, although refinement of the hazard assessment may or may not be needed.

Tier 2 Exposure and Hazard Assessments

Tier 2. Use of “market basket” exposure data and refined potency factors.



Tier 2 Exposure Assessment:

At Tier 2, refinement of potential exposure could include use of actual measured data. For the NMC insecticides, or for any group of food-use pesticides, source data could include that available from the USDA Pesticide Data Program (USDA PDP) on detection of pesticide residues and metabolites in dietary components. In an original evaluation of cumulative risk, this assessment would be conducted before moving on to probabilistic estimates of exposure. However, as an existing assessment (US EPA cumulative risk assessment of the NMC insecticides) is being used as a data source, the probabilistic exposure assessments generated in that assessment will be used in this document. This should not be taken as an indication that such highly refined estimates of exposure are called for in all cases; this is simply a use of existing data in order to test the WHO / IPCS framework.

Tier 2 Hazard Assessment:

Until the Tier 3 exposure assessment is tested against the refined potency estimates derived in Tier 1 of the hazard assessment, no further refinement of the potency or grouping based on mode of action will be

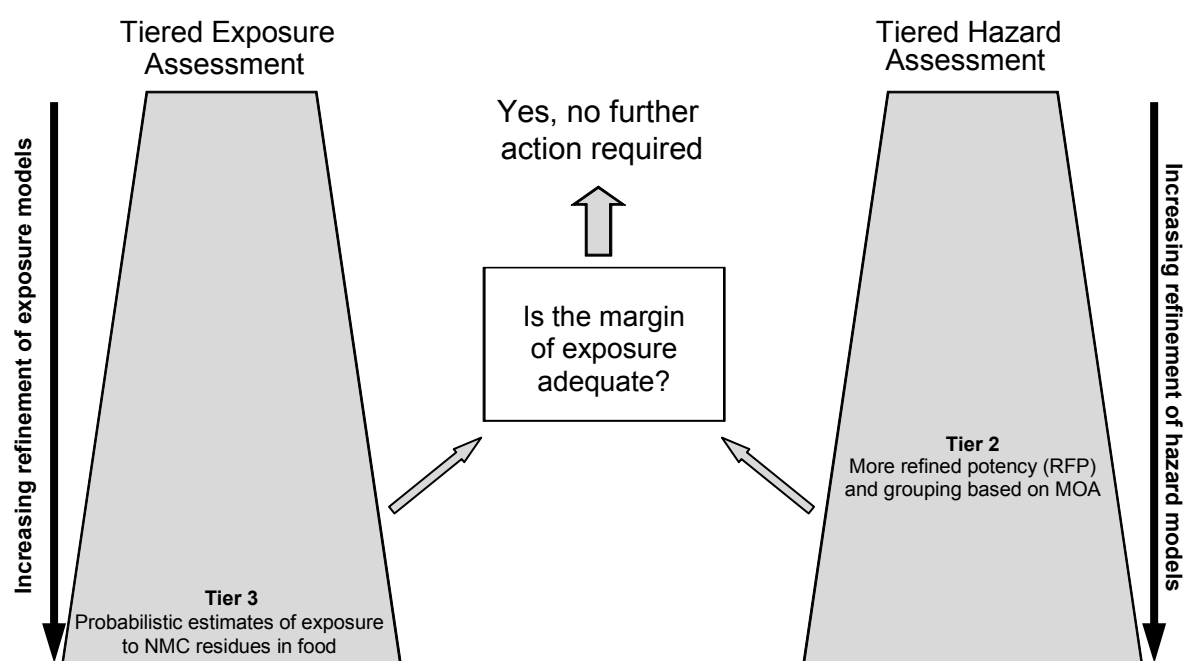
conducted. Clearly, if the Tier 1 refinement of hazard assessment is not sufficient, additional refinements will be conducted.

Summary, Tier 2 Exposure and Hazard Assessment

Ordinarily, a complete tier of both exposure and hazard assessment would not be omitted. However, as the data on which this assessment is based begin with very generic exposure estimates and move directly to probabilistic exposure assessment of dietary intake, the exposure assessment provided for at this tier will be skipped. Likewise, hazard assessment will not be further refined until it is clear whether such refinement is needed or not.

Tier 3 Exposure and Hazard Assessment

Tier 3. Probabilistic exposure modeling **Tier 2. Refinement of individual potency factors**



Tier 3 Exposure Assessment:

Food consumption data used in the cumulative risk assessment for NMCs was obtained from the United States Department of Agriculture (USDA) food consumption survey in which dietary recall data was collected from over 20,000 people ranging from infants less than one year old to adults over 50 years old. Actual residue information for each of the compounds was obtained from the USDA pesticide data program (for example, USDA, 2007), which analyzes samples of domestic and imported food at distribution centers. Unlike the data used in Tier 0 or 1, the PDP data represents residue levels after the food item is processed for consumption, shortly before purchase by the consumer, and thus is a more realistic picture of actual exposure.

Monte-Carlo simulations for exposure to the NMC compounds were conducted for each age group using dietary consumption data and pesticide residue data from the above-mentioned USDA programs. Residue

concentrations for each compound were expressed as equivalents to the index chemical, rather than as residues of each specific compound. The margin of exposure for cumulative exposure to the NMCs was then determined at the 95th, 99th, and 99.9th percentile for each age group.

Table 7. Probabilistic analysis of cumulative dietary exposure to NMC insecticides and the resulting margin of exposure

Age group	95 th percentile		99 th percentile		99.9 th percentile	
	Mg/kg bw/day	MOE	Mg/kg bw/day	MOE	Mg/kg bw/day	MOE
General population	0.0004	404	0.0023	79	0.0115	15
Infants < 1 year	0.0005	342	0.0024	74	0.0106	16
Children 1-2 yrs	0.0013	141	0.0051	35	0.0229	7.9
Children 3-5 yrs	0.0010	185	0.0044	40	0.0209	8.6
Children 6-12 yrs	0.0006	323	0.0028	63	0.0145	12
Youth 13-19 yrs	0.0003	576	0.0017	106	0.0098	18
Adults 20-49 yrs	0.0001	1278	0.0008	236	0.0042	42
Adults 50+ yrs	0.0002	1035	0.0009	193	0.0044	40
Females 13-49 yrs	0.0004	505	0.0019	97	0.0101	17

Although predicted exposure of children between 1 and 5 years of age exceeds the margin of exposure of 10 at the 99.9th percentile level of protection, for these age groups the margin of exposure is equal to 10 at the 99.8th percentile. The data tabulated above are considered by the US EPA to not indicate any concern for cumulative dietary exposure to N-methyl carbamate insecticides. This safety finding was supported, in part, based on extensive sensitivity analyses conducted on assumptions, data, and models used in the cumulative risk assessment.

Tier 2-3 Hazard Assessment:

As the cumulative risk assessment passes with Tier 2 exposure assessment and Tier 1 hazard assessment data, there is no need to further refine the hazard assessment in either Tier 2 or 3.

Summary, Tier 3 Exposure and Hazard Assessment

Because the compounds examined here share a common mechanism of action but have differing potencies, an index chemical was selected and relative potency factors for each of the other substances was derived compared to that index chemical using a highly refined meta-analysis BMD approach. Probabilistic estimates of exposure were determined and margins of exposure were determined for various age groups. This highly refined analysis demonstrates that there is no concern for cumulative dietary exposure to N-methyl carbamate insecticides.

Conclusions

Refinement of the hazard assessment by deriving relative potency factors and inter-species assessment factors, and of the exposure assessment by using realistic values for pesticide residues in food consumed, results in an exposure assessment in which cumulative dietary exposure to the NMC insecticides does not raise concerns for any age group.

Based on this, further refinement of either hazard or exposure as provided for in the WHO / IPCS framework is unnecessary. However, such refinements could be conducted in other cases where cumulative dietary exposure to pesticide residues exceeds the margin of exposure.

If the margins of exposure had not been adequate with the NMCs considered here, further refinements of the hazard assessment might include:

- conducting comparative cholinesterase inhibition assays in juvenile and adult animals to reduce the default FQPA safety factor of 10 on additional NMCs; or
- using pharmacologically-based pharmacokinetic (PBPK) modeling, given the rapid nature of onset of inhibition and rapid recovery, to better evaluate the dynamic nature of exposure to and effect of NMC insecticides.

The exposure assessment presented above actually “uses up” the highest tier listed, where probabilistic exposure estimates are provided for. However, even the scenarios listed above could be refined if the margins of exposure were not sufficient. The probabilistic exposure models used in the exposure assessment totaled consumption of all foods during a 24-hour period, rather than separating them into meals or eating events. With compounds which have a rapidly reversible biological effect such as NMC-based acetylcholinesterase inhibition, it is possible that the inhibition from NMC consumption at one meal would be reversed before the subsequent meal and potential NMC consumption. Thus evaluation of the contribution of each meal to the total daily NMC consumption might have been used to potentially reduce apparent exposure to NMCs – consumption at breakfast of sufficient NMC equivalents to the index chemical to cause 20% acetylcholinesterase inhibition at breakfast followed by consumption at dinner of NMC equivalents to cause 30% inhibition differs in predicted hazard from consumption in one 24-hour period of sufficient NMC equivalents to inhibit enzyme activity by 50%. To the extent possible with available models, US EPA has evaluated the within-day exposures to NMCs through food. This analysis is beyond the scope of this case study.

In conclusion, use of the WHO / IPCS framework for cumulative risk assessment of food-use pesticides is generally productive. The Hazard Assessment Tiers are fairly easily adapted to the available data or to that which can be generated specifically for the purpose of the cumulative risk assessment. The Exposure Assessment Tiers are less neatly divided, as registration of an agrochemical pesticide generally requires fairly refined data which may go as far as Tier 3. However, the overall approach is still applicable.

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<http://www.ams.usda.gov/AMSV1.0/ams.fetchTemplateData.do?template=TemplateG&topNav=&leftNav=ScienceandLaboratories&page=PDPDownloadData/Reports&description=Download+PDP+Data/Reports&acct=pestcddataprg>
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Session A: WHO Combined Exposures Framework: Tier 0 Assessments

Susan Felter, Procter & Gamble, Central Product Safety
On behalf of the HESI Mixtures Project Committee

WHO OECD ILSI/HESI International Workshop on
Risk Assessment of Combined Exposures to Multiple Chemicals

Paris, France
Feb 15-16, 2011



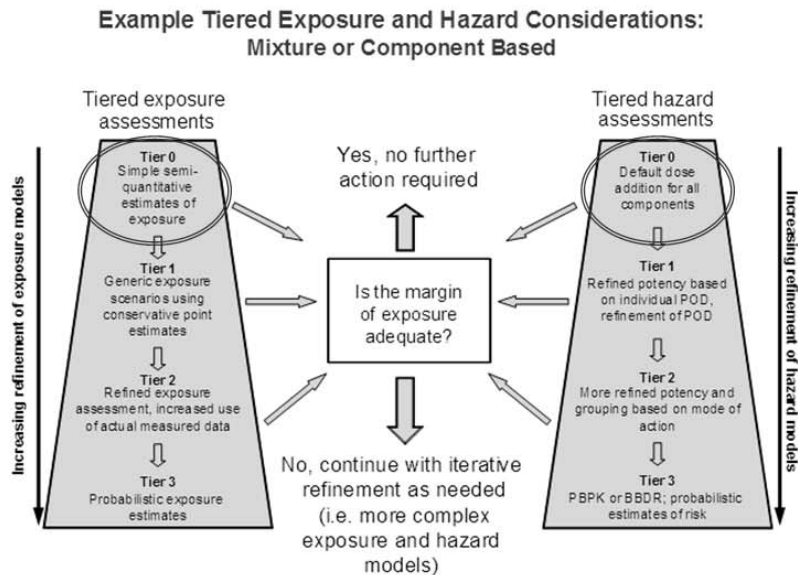
The WHO/IPCS Framework. Step 1: Problem Formulation

Problem Formulation for Combined Exposure Assessment

- *What is the nature of exposure?*
- *Is exposure likely, taking into account the context?*
- *Is there a likelihood of co-exposure within a relevant timeframe?*
- *What is the rationale for considering compounds in an assessment group?*



The WHO/IPCS Framework



3

HESI Mixtures project

- **Objective:** How can environmental chemical mixtures for which a more in-depth risk assessment is warranted be prioritized relative to those expected to be of lesser concern?
- **Strategy:** Examine utility of the Threshold of Toxicological Concern (TTC) concept to chemical mixtures as a screening-level, prioritization tool (i.e., Tier 0)
 - TTC proposes that a *de minimis* value for toxicity can be identified for many chemicals
 - When structural data are available, this is used to identify the relevant TTC (in the absence of such data, worst case assumptions can be made)

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Chemicals with alerts for genetox / DNA reactivity
 TTC = 0.15 ug/day

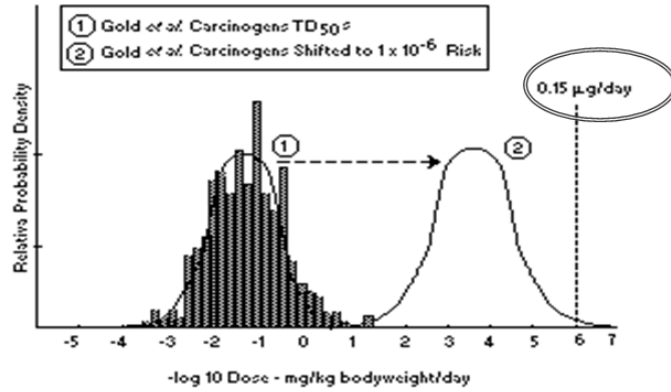
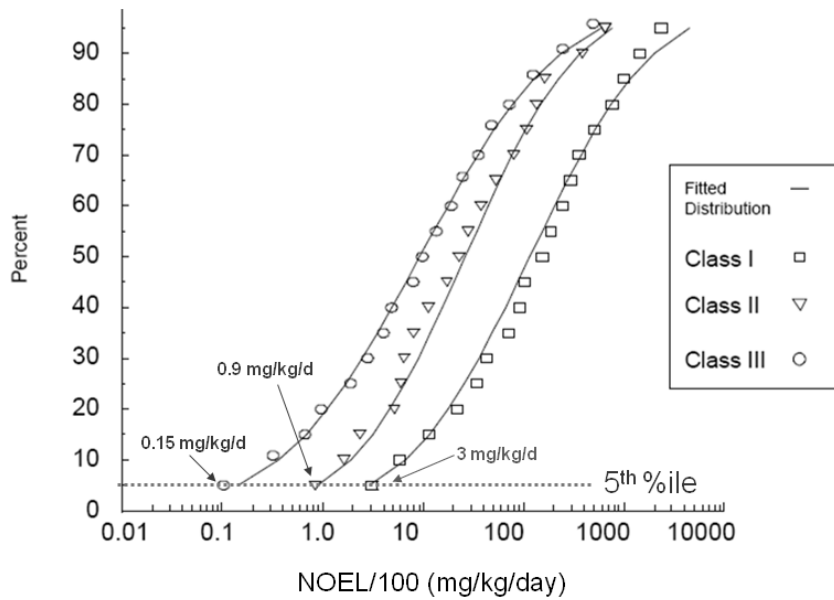


Figure 4. Distribution of TD₅₀s for 343 rodent carcinogens from the Gold *et al.* (1984) Carcinogen Potency Database and distribution of 1 x 10⁻⁶ risks calculated by linear extrapolation from the TD₅₀ values (modified from Rulis, 1989)

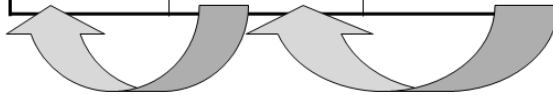
5

Threshold of Toxicological Concern (TTC)
 Noncancer Endpoints



TTC Exposure Based Limits

TTC Tier (ug/d)	Equivalent (mg/kg/d)	Basis
0.15	0.0000025	Structural alerts for genotox
1.5	0.000025	
18	0.0003	Organophosphate
90	0.0015	CC III: 5 th %ile NOEL = 0.15 mg/kg/day
540	0.009	CC II: 5 th %ile NOEL = 0.9 mg/kg/day
1800	0.03	CC I: 5 th %ile NOEL = 3 mg/kg/day



Convert from mg to ug; multiply by bw of 60 kg

Divide by UF of 100

7

Case study: Surface Water Contaminants

- 10 substances found in surface waters
- Multiple health-protective assumptions
 - Assume all present simultaneously at all times, at maximum concentration detected
 - Assume all belong to same assessment group (supporting assumption of dose addition)
 - Assume 100% of drinking water from this source
 - Maximum exposure will be in children of 3-6 years of age.
 - Exposure (mg/kg/d) calculation:

$$\frac{\text{Surface water conc. (ppm)} * 0.42 \text{ L consumption/ day}}{18 \text{ kg body weight}}$$

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Assignment of Cramer Class & TTC Value

Compound	Water conc [ppb]	Exposure (mg/kg/d)	Cramer class	TTC (mg/kg/d)
A	0.083	1.94E-06	II	0.009
B	0.076	1.77E-06	III	0.0015
C	3.8	8.87E-05	II	0.009
D	1.7	3.97E-05	I	0.03
E	0.13	3.03E-06	III	0.0015
F	0.18	4.20E-06	III	0.0015
G	34	7.93E-04	II	0.009
H	0.28	6.53E-06	I	0.03
I	6.1	1.42E-04	III	0.0015
J	1.1	2.57E-05	I	0.03

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Calculating the Hazard Quotient (HQ) and Hazard Index (HI)

- $$HQ_{\text{individual substance}} = \frac{\text{Exposure}_{\text{individual substance}} \text{ (mg/kg-bw/day)}}{\text{TTC value}_{\text{individual substance}} \text{ (mg/kg-bw/day)}}$$

- $$HI_{\text{mixture}} = HQ_A + HQ_B + HQ_C + HQ_D \dots + HQ_J$$

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Case Study: HQ's and HI for Mixture

Compound	Exposure (mg/kg/d)	TTC (mg/kg/d)	HQ based on TTC
A	1.94E-06	0.009	0.00021
B	1.77E-06	0.0015	0.0012
C	8.87E-05	0.009	0.0097
D	3.97E-05	0.03	0.0013
E	3.03E-06	0.0015	0.0020
F	4.20E-06	0.0015	0.0028
G	7.93E-04	0.009	0.0870
H	6.53E-06	0.03	0.00024
I	1.42E-04	0.0015	0.0950
J	2.57E-05	0.03	0.00086
Hazard Index			0.200

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TTC and mixtures: Key questions

- How can mixtures be evaluated where chemical structures are unknown?
- How should mixtures that contain chemicals in different Cramer classes be dealt with?
- What is the potential for interactions (e.g., synergy) in multi-component mixtures?

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How should mixtures that contain chemicals in different Cramer classes be dealt with?

- Low Dose, Component-Based Approaches
 - Additive models:
 - Dose-additive
 - Response-additive
 - Require the assumption that synergy does not occur

or

- The magnitude of any interaction is such that synergy can be taken into account in the default assumptions in the RA

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Synergy literature review

- **Key question:** What is the maximum extent to which additive models would underestimate the toxicity of a mixture at exposure levels of interest?
- **Review included:**
 - Synergy in mammalian test systems at low doses
 - Key literature 1990 – present; references prior to 1990 identified from reviews and databases
 - Call for papers (HESI, ECETOC, IPCS, SOT mixtures specialty group)
- **Data sources:**
 - Scientific journal databases (PubMed/MEDLINE; TOXLINE; Google Scholar; SCIRUS); ATSDR Interaction Profiles; EPA's MIXTOX database; OSHA website

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Search methodology

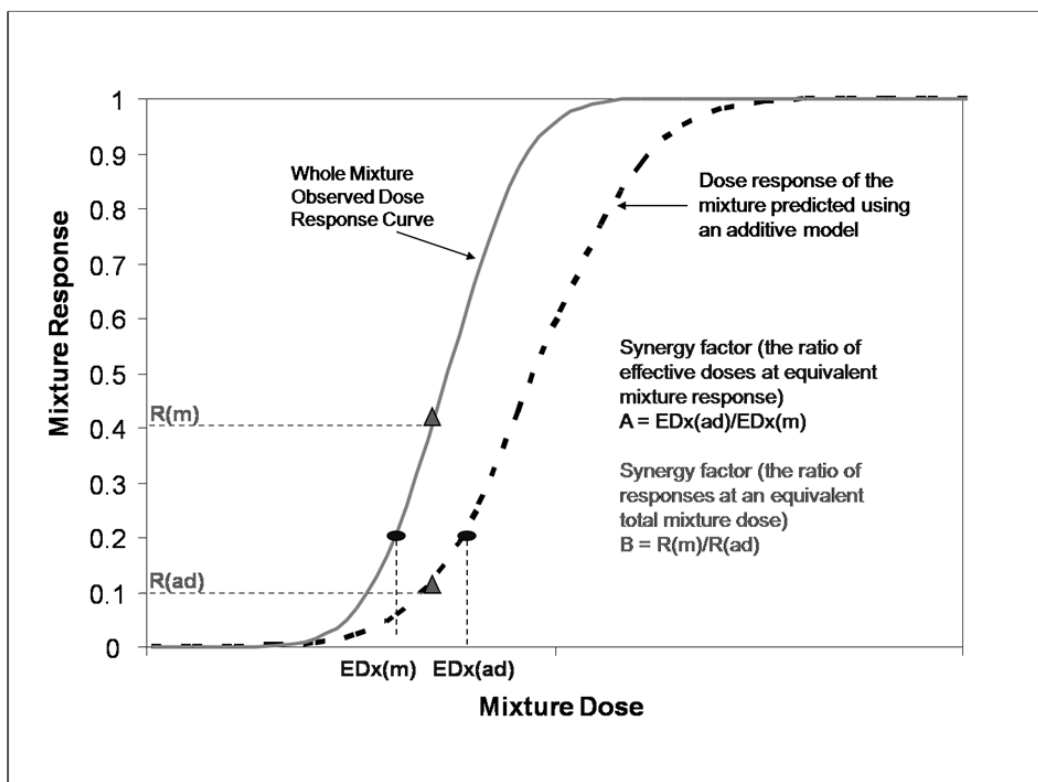
- “Low dose” defined as a dose near the POD (e.g., NOAELs, BMDLs, etc) of the chronic health-based guidance values of individual mixture components
 - Expanded to include shorter term studies of sub-lethal endpoints, even if doses exceeded chronic PODs
- Only included studies reporting positive interactions (no consideration given to antagonistic interactions or studies reporting no interactions)
- Excluded studies lacking defined standards for data quality

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Definition and Evaluation of Synergy

- For this review, synergy was defined as a mixture response that exceeds that predicted from the no-interaction model (the determination that a dose- or response-additive model will underestimate a mixture’s toxicity)
- Two methods are commonly used in the literature to measure synergy
 - Decrease in dose required to produce a specific response [A]
 - Increase in response at a specific dose of a mixture [B]

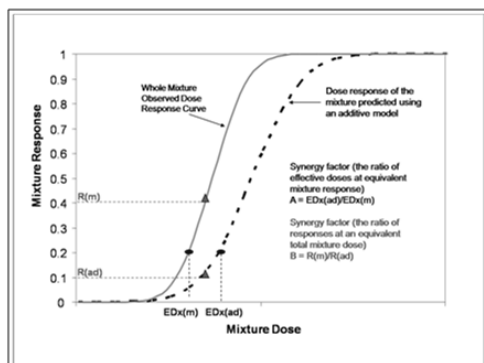
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Evaluation of synergy

- [A] Decrease in dose required to produce a specific response
- [B] Increase in response at a specific dose of a mixture

- Applying [A] and [B] to the same experimental findings gives different values for the magnitude of synergy
- Method [A] is the preferred method because it better relates to the risk assessment process (changes the point of departure) and because it has better mathematical properties

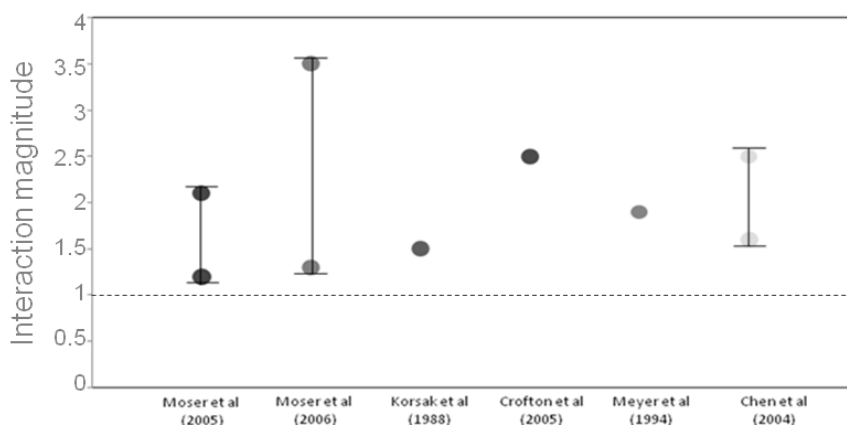


Results

- Literature search identified 90 potentially relevant papers, with data on 204 unique chemicals, which were critically evaluated
- Data from all 90 papers were entered into a database
- Excluding studies that did not provide novel findings on synergy and those where synergy was not observed, 43 papers remained, with original mammalian data from which synergy for 126 chemicals could be examined
 - Duration of dosing (no. of studies) : short term>sub-acute>sub-chronic>chronic
- Positive interaction magnitudes were reported in 11 of the studies (though not at all dose levels)
- Five studies were excluded from the final analysis based upon critical evaluation (e.g., methodological issues; endpoint was toxicokinetic vs toxicity)

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(Potential) Synergy Values Reported



Route	oral	oral	inhalation	oral	dermal	multi
Species	rat	rat	rat	rat	mouse	human
Endpoint	ED20 – motor activity, gait score, ChE levels	ED20 – motor activity, gait score, ChE levels	ED50 – rotarod performance	ED30 – serum T4 levels	# skin tumours	Lung tumour incidence

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Conclusions

- The maximum potential magnitude of synergy did not appear to exceed ~4-fold for studies meeting the review criteria
- *So* -- There is merit in the conventional wisdom that toxicological interactions are not likely to occur at the low doses permitted under existing standards for chronic exposure, including those for cumulative stressors.
- *However* -- Insufficient data were obtained to support robust conclusions regarding potential or not for synergy following low-dose, chronic exposures
 - Of 90 studies identified, only six (6) reported a quantified value for [potential] synergy (with many of these showing synergy only at highest doses tested)
 - Considerable variation in methodology used
 - In most studies, the dose of mixture components exceeded the POD for the individual components

Boobis *et al* (**in press**). Critical analysis of literature on low dose synergy for use in screening chemical mixtures for risk assessment. *Crit. Rev. Toxicol.*

Remaining Questions & Implications for a TTC-Based Mixtures Screening Approach:

- How should synergy be expressed/calculated (can we agree upon a methodology?)
- How do exposure levels compare to TTC thresholds? To effect thresholds?
- Are there any cases where synergy occurs at low dose but differs from known high dose synergy patterns?
- Need information of how often synergy is/is not observed?
- Where synergy is observed, what is toxicological significance (e.g. consider endpoints, dose used, exposure duration, magnitude relative to overall uncertainty)

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Case Study: Surface Water Monitoring Data

Application of TTC Approach to
Evaluate Mixtures Toxicity

February 15, 2011
WHO, Paris

Purpose and Approach

Illustrate real-world application of TTC in the
assessment of chemical mixtures found in
surface waters.

Exposure Assumptions

- Exposure to general population was via direct consumption of surface water through drinking
 - Simultaneously
 - Continuously
 - Worst case scenario considered
- All conservative assumptions intentionally selected, including dose additivity

Levels of Contaminants in Surface Waters

Hazard Assessment

- Approx. 1/3 of chemicals lacked readily available toxicity data
- 34/48 samples included ≥ 1 chemicals with no toxicity standard, thus 70% of the samples could not be assessed for toxicity
- Cramer classification of each chemical was performed

Cramer Classification of Surface Water Contaminants

- 60 % (29/48) samples POD based on Cramer Classification
- Only 40% (19/48) PODs based on chemical specific data
- Minimal

Cramer Classification of Surface Water Contaminants

List of all Cramer Classification, TTC values and pertinent information for all Surface Water Contaminants ?

Method: Real World Mixture

- 48 mixtures evaluated consisted of 2 to 23 components
- Mixture components involved 45 unique structures that fall into various chemical classes, such as fragrances, pesticides, surfactants, personal care products, solvents, and petrochemicals
- 4-octylphenol (II), bromoform (III), caffeine (III), cholesterol (I), diazinon (III), fluoranthene (II), phenol (I), pyrene (III), toluene (I), triclosan (III)
- Representative of the environment and included a wide range of chemicals with differing levels of toxicity

Hazard Quotient and Index

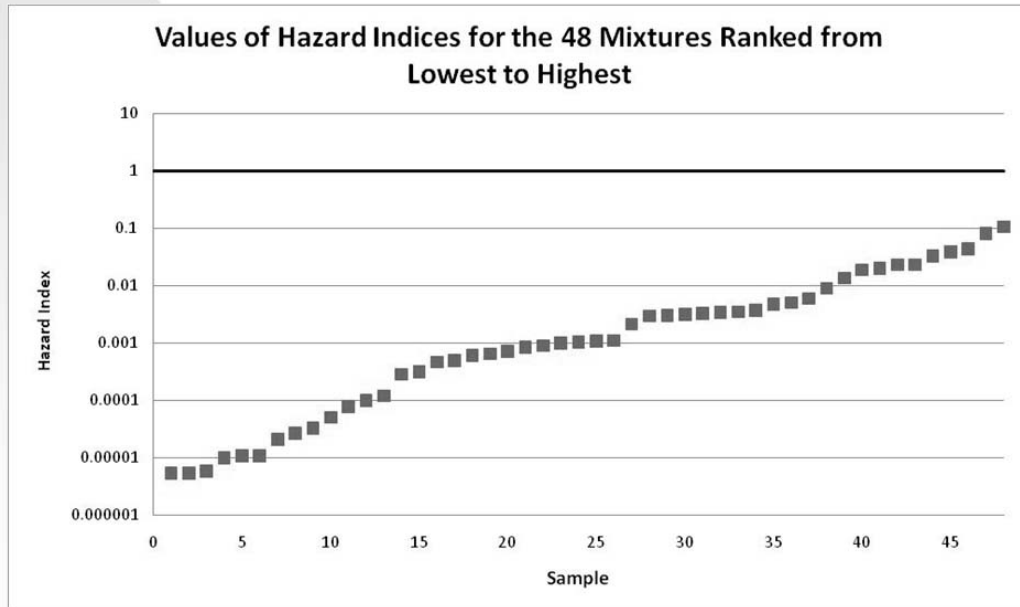
$$HQ_{\text{individual substance}} = \frac{\text{Exposure}_{\text{individual substance}} \text{ (mg/kg body weight per day)}}{\text{TTC value}_{\text{individual substance}} \text{ (mg/kg body weight per day)}}$$

$$HI_{\text{mixture}} = HQ_A + HQ_B + HQ_C + HQ_D \dots + HQ_J$$

Hazard Index Calculations

Should we give the HQ/HI calculations for all Surface Water contaminants?

Results



Results

- **For Adults:** HIs were calculated based on 70 kg body weight and default water intake of 2 liters/day. All values were below 0.2, indicating minimal concern for mixtures toxicity
- **For Infants:** HIs would be 3.5x, for 10 kg body weight infants using default water intake of 1 liter/day. Still all HIs will be <1, indicating minimal concern for mixtures toxicity.
- **Built in conservatism:** 48 mixtures were from grab samples of surface water, not drinking water.

Are Hazard Indices based on the TTC Conservative?

- The data set allows the impact of using the TTC to be tested
- 15 of the 48 mixtures have toxicity data on all of the components
- The TTC approach was used to estimate the Hazard Indices of the 15 mixtures and the results are compared to the actual Hazard Indices

Use of TTC results in larger Hazard Indices

Hazard Index Based on Cramer Class (TTC)	Hazard Index Based on Compound- Specific Toxicity	Ratio
0.0039	0.00090	4.3
0.00057	0.000063	9.0
0.011	0.00088	12
0.0037	0.00026	14
0.00072	0.000041	18
0.0019	0.000082	23
0.00080	0.000022	36
0.00067	0.000017	39
0.0086	0.000098	88
0.00073	0.0000047	150
0.00075	0.0000044	170
0.0020	0.0000089	230
0.0093	0.000027	340
0.042	0.0000081	5200
0.052	0.0000088	5900

Chemical Interactions

Use of the TTC will over estimate toxicity of most mixtures.

As a result mixtures that pass a TTC based tier 0 are likely to be well below any threshold of interaction between any of the mixture components.

Conclusions

The use of the TTC to estimate the contribution from chemicals with out toxicity data to mixtures can be a useful Tier 0 tool.

The use of TTC worked in this example because the chemicals present in the samples of surface water were present at very low concentrations

The mixtures HIs of mixtures were all below 0.2

The interactions among the mixtures at such low levels (ppb range) are believed to be negligible.

Case study to illustrate real-world application of TTC in the assessment of chemical mixtures actually found in surface water

[Text to accompany the Powerpoint presentation of the case study]

Prepared by: The ILSI HESI Mixtures Project Committee

1. Introduction

Surface water represents a real-world example of a set of complex mixtures that are composed of low level concentrations of a diverse range of substances representing various chemical classes. The mixtures present an additional challenge to risk assessors since many of the identified chemicals have limited toxicity data that do not support the derivation of chronic health standards or health-based guidance values. In fact, for several components of such mixtures there is no information to evaluate their toxicity.

The intent of this case study is to illustrate the potential utility of applying the threshold of toxicological concern (TTC) approach in a Tier 0 assessment to prioritize the need for further evaluation of a chemical mixture. This case study applies the traditional toxicity assessment system as well as the Cramer et al. (1978) system that classifies individual chemical components of a mixture into three classes based on consideration of systemic toxicity that is then used in the TTC approach. The study assumes that the only information available is 1) the identities and structures of the chemicals in the mixture and 2) their respective levels in surface water. Based on these two pieces of information, it should be possible to use the TTC approach (Kroes et al., 2004) as a Tier 0 assessment tool.

In principle, the TTC approach sets a *de minimis* value below which exposure is considered unlikely to be a concern, based upon structural characteristics of the candidate chemical and existing toxicity data for other substances in an identified database. This database was developed by Munro et al. (1996) and draws upon work by Cramer et al. (1978) that divides low molecular weight organic chemicals into three classes by analyzing toxic, systemic toxicity (non-carcinogenic effects) according to chemical structure. Chemicals that are potentially genotoxic (on the basis of prediction or measurement) are considered a separate class, with their own TTC value. Some chemical structures are excluded *a priori* from the TTC approach, including certain potent genotoxic compounds (e.g., aflatoxin-like, azoxy- and *N*-nitroso-compounds), as well as polyhalogenated dibenzo-*p*-dioxins and dibenzofurans, steroids, proteins and nonessential metals (Kroes et al., 2004). The TTC approach has been applied to food safety assessments, and additional work is ongoing to investigate its potential application to mixtures in general, as a Tier 0 approach.

This case study is a real world application of the TTC approach to a set of 48 mixtures from Price et al. (2009). These mixtures were identified from monitoring data of contaminants found in samples of surface waters in the State of Minnesota (Lee et al., 2004). These chemicals fall into various classes, such as fragrances, pesticides, surfactants, personal care products, solvents and petrochemicals found routinely in wastewater discharges (see Table 1).

This case study assumes dose addition, where all components are considered to contribute to the toxicity of the mixture. However, this approach does not explicitly address the possibility of synergy. As discussed in the WHO/IPCS framework document, the use of dose additivity is considered conservative based on analysis of empirical results for effects of combined exposure including to chemicals that induce critical effects by different modes of action (ATSDR, 2001; U.S. EPA, 2007; EFSA, 2008; European Commission, 2010).

In a recent exercise, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) performed a literature review focused on findings of synergy at or near doses associated with a study's points of departure (e.g., the benchmark dose [BMD], no-observed-effect level [NOEL], etc.) that may be associated with the development of chronic health-based guidance values of individual mixture components. This review found few studies that reported a quantified value for synergy at the low doses used; from those studies, the maximum potential magnitude of synergy did not exceed approximately four-fold (e.g., less than four times that predicted based on an assumption of dose addition). However, most of the studies included in the review were performed at doses that caused overt toxic effects from acute exposures (Embry et al., 2009; Boobis et al., (Critical Reviews Toxicology, accepted for publication). The additive model is expected to exclude exposures to such levels.

As a Tier 0 case study, the study design was such that the emphasis was not on developing highly certain quantitative estimates of risk, but rather to utilize a conservative approach to demonstrate safety (defined as $HI < 1$) with a low chance of false negatives. This screening-level approach demonstrates a more efficient approach that would obviate the need for a more detailed, resource-intensive, higher-tier evaluation. Assumptions were intentionally selected to be conservative – i.e., to result in a hazard index (HI) that would overestimate rather than underestimate risk for a chronic lifetime exposure via drinking water ingestion. As demonstrated below, the use of the Cramer classes is conservative and will tend to over estimate values of HI. In the exposure portion of the assessment we also made conservative assumptions. Specifically, we assumed that each mixture reflected a combination of chemicals that would occur over long periods of time. In addition, the surface water was assumed to be used as drinking water and that the chemicals were not removed or diluted during the treatment of the drinking water. Finally, we assumed that the individual would receive all of their water for long periods of time from this single surface water source.

2. Considering the need for a framework analysis

The World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) framework for the risk assessment of combined exposure to multiple chemicals presents a series of questions designed to determine whether it is appropriate to group substances in an assessment group for, in this example, a Tier 0 screening-level risk assessment.

- *What is the nature of exposure? Are the key components known? Are there data available on the hazard of the mixture itself?*

The purpose of the Tier 0 assessment is to serve as an initial screening-level approach, to aid prioritization of those mixtures that should be subject to higher-tier assessments (e.g., Tiers 1, 2 and 3) and differentiate those that are expected to be of lesser concern. This tier is intended to be conservative and often relies on

semi-quantitative estimates of exposure based on very limited data. Human exposure via consumption of the water was assumed to be the completed exposure pathway.

- *Is exposure likely, taking into account the context?*

The exposure of the general population is assumed to occur via direct consumption of surface water as drinking water. This assumption was made for the purpose of assessing the potential of the mixtures to pose a risk. As discussed above, actual exposures are likely to be lower than the assumptions made in this assessment.

- *Is there a likelihood of co-exposure within a relevant timeframe?*

These mixtures were actually identified in grab samples of surface waters. If the water is consumed than exposures will co-occur.

- *What is the rationale for considering compounds in an assessment group?*

As part of the Tier 0 assessment a worse case assumption is made that all of the chemicals in the mixtures fall into one assessment group. This would be revisited in higher tiered assessments if necessary.

3. Purpose and focus of the assessment

This case study addresses a Tier 0 screening-level risk assessment for chemicals identified in surface waters to determine whether there is a possible human health concern from the consumption of such water.

4. The framework analysis

4.1. Tier 0

4.1.1. Exposure assessment

Concentrations of the 48 mixtures are provided in the appendix to this paper. The concentrations are obtained from actual monitoring data, wherein 44 unique chemicals were identified. The following assumptions were made regarding this exposure:

- One hundred percent of drinking water consumption comes from this single source.
- Exposure values for children via drinking water are used given the higher exposure per kilogram body weight for children compared with adults as a conservative assumption. These values are estimated based on average consumption of 0.42 L of water per day by an 18 kg child (aged 3–6 years) (U.S. EPA, 2008):

$$\text{Exposure (mg/kg body weight per day)} = \frac{\text{Surface water concentration (mg/L)} \times 0.42 \text{ L/day}}{18 \text{ kg}}$$

4.1.2. Hazard assessment

This Tier 0 example assumes that the toxicological information on the 44 chemicals varies. For some chemicals, large data bases of toxicity are available whereas for other chemicals, no toxicity data are available. The latter case occurs for 13 of the 44 chemicals. The appropriate Cramer class was determined for each of the 13 chemicals using ToxTree version 1.60 (<http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE>) (Table 1). For the 31 chemicals where toxicity data were available, toxicity values were developed using the recent guidance for setting DNELs (ECHA, 2008).

The TTC approach relies on conservative daily exposure thresholds for each Cramer class, derived from a database of subchronic, chronic and reproductive/developmental oral toxicity data on more than 600 chemicals (Munro et al., 1996). The most conservative NOEL values were plotted based on structural class, and values corresponding to the 5th percentile NOEL were selected. The 5th percentile NOELs were then multiplied by 60 kg (average adult body weight) and divided by an uncertainty factor of 100 (an additional 3X uncertainty factor was applied to subchronic NOELs) to calculate the following human exposure threshold (i.e., TTC) values:

- 1800 µg/day (Cramer class I)
- 540 µg/day (Cramer class II)
- 90 µg/day (Cramer class III)

To use these TTC values for comparison with exposures of populations other than adults, the values should be converted back into units of mg/kg/day. Accordingly, Cramer class I chemicals have human exposure threshold values of 0.03 mg/kg/day; class II, 0.009 mg/kg/day; and class III, 0.0015 mg/kg/day (Barlow, 2005).

4.1.3. Risk characterization/analysis of uncertainties

4.1.3.1 Hazard Index

Hazard quotients (HQ) for each mixture component can be calculated by dividing the exposure values by the respective TTC value:

$$HQ_{\text{individual substance}} = \frac{\text{Dose}_{\text{individual substance}} (\text{mg/kg/day})}{\text{Toxicity value (actual data or TTC value)}_{\text{individual substance}} (\text{mg/kg/day})}$$

A hazard index (HI) for the mixture can be calculated, assuming dose addition for all components, by taking the sum of the individual hazard quotients:

$$HI_{\text{mixture}} = HQ_A + HQ_B + HQ_C + HQ_D \dots + HQ_I$$

Higher-tier assessment would not be a high priority if the hazard index is less than 1. Utilizing the hazard index in this way corrects exposure values based on estimates of chemical potency using Cramer classification. As shown in Figure 1, all HI values were below 0.2. If any of the HIs were ≥ 1 , this would indicate that the assessment should be further refined. In this case, the results of the Tier 0 assessment suggest that advancement to higher assessment tiers is not necessary in this case.

As stated above, assumptions were intentionally selected to be conservative—i.e., to result in a hazard index that would overestimate rather than underestimate risk for a chronic lifetime exposure via drinking water ingestion:

- All drinking water was assumed to be from the single source containing all contaminants identified (see Section 4.1.1). This was a conservative approach and does not consider that people generally obtain water from more than one source.
- On a per kilogram body weight basis, children have greater water consumption than adults (see Section 4.1.1). The average drinking water consumption rate of a child was used, which is greater than a chronic lifetime drinking water intake on a per kilogram body weight basis. Alternative consumption rates could be utilized.
- The TTC is a conservative approach. Each TTC value represents the 5th percentile NOEL of all compounds in the dataset for that particular class (i.e., 95% of the compounds in the class were more toxic), with uncertainty factors of 100 applied. This case study utilized the TTC value for each mixture component and assumed dose addition for all Cramer classes (rather than independence).
- The use of dose additivity is considered conservative based on analysis of empirical results for effects of combined exposure including to chemicals that induce critical effects by different modes of action (see Section 1).

4.1.3.2 Using Hazard Indices Based on Chemical Specific Toxicity to Demonstrate the Conservative Nature of the Cramer Class Approach

As discussed above, 15 of the mixtures have toxicity values for all of the chemicals in the mixture. Thus the Cramer class approach was not required to evaluate these 15 mixtures. Therefore, these 15 mixtures provide an opportunity to test how conservative the toxicity values are that are produced by the Cramer class approach. This test was performed by using the Cramer classes to estimate the toxicity of the chemicals in these 15 mixtures. The HQ values were determined and summed to give the HI values. The Cramer class based estimates of HI for the 15 mixtures were then compared to the HI values that were based on actual toxicity data. The ratios of the two estimates of the 15 HI values are given in Table 2. As Table 2 indicates, the use of the TTC produced HI values that were 4 to 5900 fold higher than the values that were based on actual toxicity data. This finding suggests that the Cramer class approach is likely to produce conservative estimates of toxicity for the mixtures.

5. Conclusions

This real world case study demonstrates the potential utility of applying the TTC approach as a Tier 0 assessment screening tool for chemical co-exposures. But additional analyses with other Tier 0 chemicals should be conducted to draw generic conclusions.

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Table 1.

Compound	Cramer Class	Test species for of Point departure	Point of Departure [mg/kg/d]	UF inter-species	UF intra-species	UF duration	UF LOEL to NOEL	UF lack of data	Toxicity Based Standard mg/kg/d	TTC based Estimate mg/kg/d	Final Std mg/kg/d
1-methylnaphthalene	III	Mouse	71.6	17.5	10	1	3	1	0.14	0.0015	0.14
2-methylnaphthalene	III	Mouse	3.5	17.5	10	1	1	1	0.02	0.0015	0.02
3-beta-coprostanol	III			10	10	1	1			0.0015	0.0015
3-methyl-1H-indole (skatol)	III			10	10	1	1			0.0015	0.0015
4-octylphenol	II			10	10	1	1			0.0091	0.0091
5-methyl-1H-benzotriazole	III			10	10	1	1			0.0015	0.0015
AHTN	II	Rat	5	10	10	2	1	1	0.025	0.0091	0.025
Anthracene	III	Mouse	1000	17.5	10	2	1	1	2.9	0.0015	2.9
Benzophenone	III	Rat	15	10	10	1	3	1	0.05	0.0015	0.05
<i>beta-sitosterol</i>	III	Rat	4000	10	10	2	1	1	20	0.0015	20
<i>beta-stigmastanol</i>	III			10	10	1	1			0.0015	0.0015
Bisphenol-A	III	Rat	50	10	10	2	1	1	0.25	0.0015	0.25
Bromacil	III	Dog	5	3.75	10	1	1	1	0.13	0.0015	0.13
Bromoform	III	Rat	17.9	10	10	2	1	1	0.09	0.0015	0.09
Caffeine	III	Rat	150	10	10	2	1	1	0.75	0.0015	0.75
Carbazole	III			10	10	1	1			0.0015	0.0015
Cholesterol	I			10	10	1	1			0.0300	0.0300
Cotinine	III			10	10	1	1			0.0015	0.0015

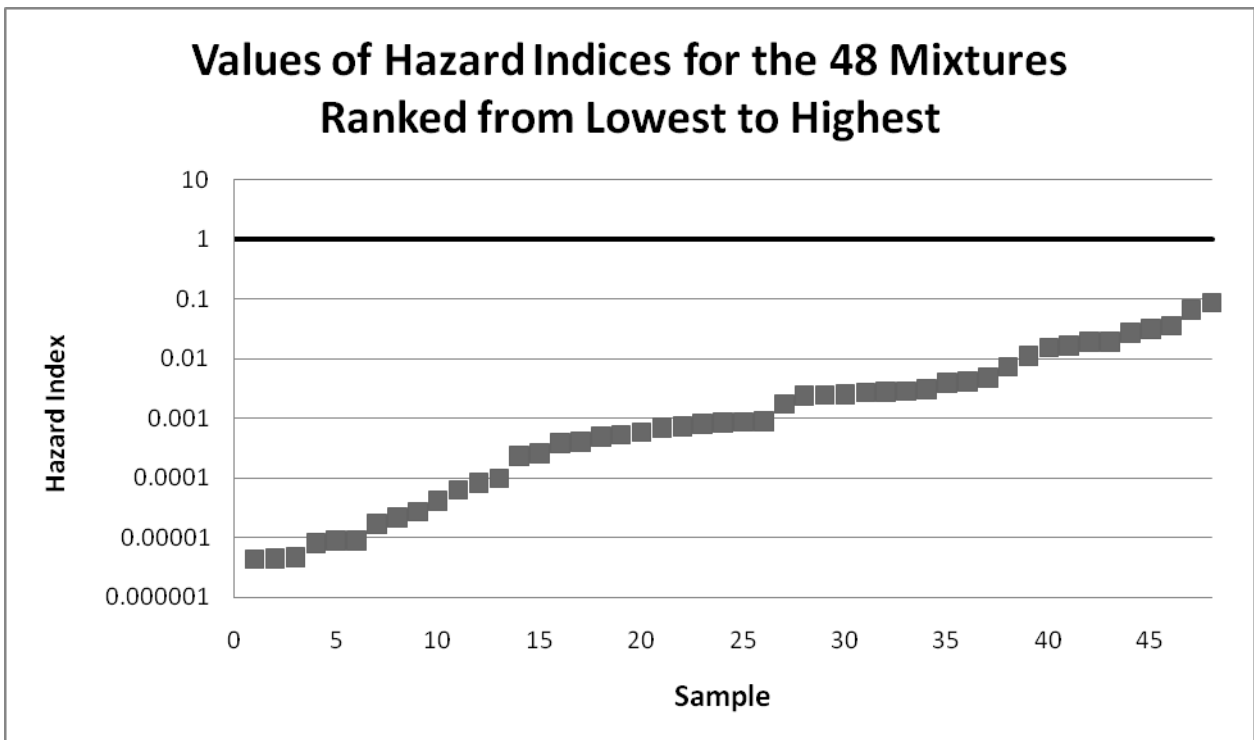
Compound	Cramer Class	Test species for of departure	Point of Departure [mg/kg/d]	UF inter-species	UF intra-species	UF duration	UF LOEL to NOEL	UF lack of data	Toxicity Based Standard mg/kg/d	TTC based Estimate mg/kg/d	Final Std mg/kg/d
Diazinon	III	Dog	0.02	3.75	10	1	1	1	0.0005	0.0015	0.0005
<i>d-limonene</i>	I	Rat	10	10	10	2	1	1	0.0500	0.0300	0.0500
Fluoranthene	II	Mouse	125	17.5	10	2	1	1	0.3571	0.0091	0.3571
HHCB (Galaxolide)	III			10	10	1	1			0.0015	0.0015
Indole	III			10	10	1	1			0.0015	0.0015
Isophorone	II	Dog	150	3.75	10	2	1	1	2.0000	0.0091	2.0000
Menthol	I	Rat	188	10	10	1	1	1	1.8800	0.0300	1.8800
Methyl salicylate	III			10	10	1	1			0.0015	0.0015
Metolachlor	III	Rat	15	10	10	1	1	1	0.1500	0.0015	0.1500
Naphthalene	III	Rat	71	10	10	2	1	1	0.3550	0.0015	0.3550
N,N-diethyl-meta-toluamide (DEET)	III	Rat	100	10	10	1	1	1	1.0000	0.0015	1.0000
Nonylphenol diethoxylate (NP2EO)	II	Rat	15	10	10	2	3	1	0.0250	0.0091	0.0250
Octylphenol diethoxylate (OP2EO)	II			10	10	1	1			0.0091	0.0091
Pentachlorophenol	III	Rat	3	10	10	1	1	1	0.0300	0.0015	0.0300
Phenanthrene	III			10	10	1	1			0.0015	0.0015
Phenol	I	Rat	93	10	10	2	1	1	0.4650	0.0300	0.4650
Prometon	III	Rat	15	10	10	2	1	1	0.0750	0.0015	0.0750
Pyrene	III	Mouse	75	17.5	10	2	1	1	0.2143	0.0015	0.2143
Tetrachloroethylene	III	Mouse	14	17.5	10	6	1	1	0.0133	0.0015	0.0133

Compound	Cramer Class	Test species for Point of departure	Point of Departure [mg/kg/d]	UF inter-species	UF intra-species	UF duration	UF LOEL to NOEL	UF lack of data	Toxicity Based Standard mg/kg/d	TTC based Estimate mg/kg/d	Final Std mg/kg/d
Tri(2-butoxyethyl)phosphate	III	Rat	100	10	10	6	1	1	0.1667	0.0015	0.1667
Tri(2-chloroethyl)phosphate	III	Rat	44	10	10	1	3	1	0.1467	0.0015	0.1467
Tributyl phosphate	III	Rat	9	10	10	1	1	1	0.0900	0.0015	0.0900
Triclosan	III	Monkey	30	5	10	1	1	1	0.6000	0.0015	0.6000
Tri(dichlorisopropyl)phosphate	III	Rat	5	10	10	1	1	1	0.0500	0.0015	0.0500
Triethyl citrate (ethyl citrate)	III	Rat	2000	10	10	1	1	1	20.0000	0.0015	20.0000
Triphenyl phosphate	III	Rat	161	10	10	2	1	1	0.81	0.0015	0.81

Table 2: Comparison of the Hazard Indices (HI) calculated for mixtures using chemical specific data versus based on TTC approach

HI Based on Cramer Class	HI Based on Actual Toxicity Data	Ratio
0.0039	0.00090	4.3
0.00057	0.000063	9.0
0.011	0.00088	12
0.0037	0.00026	14
0.00072	0.000041	18
0.0019	0.000082	23
0.00080	0.000022	36
0.00067	0.000017	39
0.0086	0.000098	88
0.00073	0.0000047	150
0.00075	0.0000044	170
0.0020	0.0000089	230
0.0093	0.000027	340
0.042	0.0000081	5200
0.052	0.0000088	5900

Figure 1



Risk Assessment of Combined Exposure to Multiple Chemicals: A WHO/IPCS Framework

Case Study – Food Additives

Introduction

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorized to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

The risk assessment in the area of Food Additives and Food Contact Materials often deals with limited available information. Therefore, quite generic approaches are used for first tier assessments. Such approaches are used both by EFSA and WHO/JECFA. In addition, additives and flavourings may be employed in combination or share common toxicological properties. At least, consumers may be exposed to various additives/flavourings in combinations. Therefore in specific cases group assessments are performed. In this document, an example of an EFSA approach is given.

Problem Formulation

Nature of the Exposure

The present Flavouring Group Evaluation deals with 37 branched- and straight-chain unsaturated carboxylic acids and esters of these with aliphatic saturated alcohols. Many of these compounds occur naturally in a variety of food products. In addition, these compounds are added to a range of food stuffs.

Likelihood of co-exposure

Since these flavouring compounds are present in such a variety of products, co-exposure through dietary intake is likely to occur.

Common Assessment Group

Because of structural similarities of these substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the metabolic pathways could be overloaded. Therefore, combined intake should be considered.

EFSA Example (excerpts of total opinion only)

Group approaches for branched- and straight-chain unsaturated carboxylic acids and esters of these with aliphatic saturated alcohols.³

The present Flavouring Group Evaluation deals with 37 branched- and straight-chain unsaturated carboxylic acids and esters of these with aliphatic saturated alcohols. Two substances possess chiral centres. Thirty-one of the 37 candidate substances can exist as geometrical isomers. For 10 of these substances [FL-no: 08.072, 08.083, 08.101, 08.119, 08.120, 09.181, 09.329, 09.335, 09.379 and 09.637] Industry has informed that they exist as a “mixture of isomers”. However, the Panel does not consider this information sufficient and requests quantitative data on the isomeric composition of these mixtures.

Exposure Assessment

The exposure to these substances is estimated by a generic approach requiring input at a high abstraction level. The Maximised Survey-derived Daily Intake (MSDI (SCF, 1999)) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the EU population⁵ (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999). In the terms of the WHO Combined Exposures Framework this is a typical example of a Tier 0 exposure assessment requiring only the production volume and the demographic information on population size.

Hazard Assessment

The hazard assessment is based principally on the concept of the Threshold for Toxicological Concern (TTC). In addition, to that the available toxicological information is used where available. Metabolism and kinetics have an important place in this assessment.

For example, the exposure estimates are first compared to the TTC for their specific Cramer classes, where necessary toxicological information (e.g. NOAELs) can be used for refined assessment.

³ SCIENTIFIC OPINION Flavouring Group Evaluation 5, Revision 2 (FGE.05Rev2): Branched- and straight-chain unsaturated carboxylic acids and esters of these with aliphatic saturated alcohols from chemical groups 1, 2, 3 and 51. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); European Food Safety Authority (EFSA), Parma, Italy. [EFSA Journal 2010; 8(10):1400].

Risk Assessment

Thirty-four candidate substances belong to structural class I while three substances were assigned to structural class II, according to the decision tree approach presented by Cramer et al., 1978. Thirty-three of the candidate substances in the present group have been reported to occur naturally in a wide range of food items. According to the default MSDI approach, the 37 candidate substances in this group have intakes in Europe from 0.0012 to 240 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day) and structural class II (540 microgram/person/day) substances.

On the basis of the reported annual production volumes in Europe (MSDI approach), the combined intake of the 34 candidate substances belonging to structural class I and of the three candidate substances belonging to structural class II would result in a total intake of approximately 290 and 0.19 microgram/*capita*/day, respectively. These values are lower than the thresholds of concern for structural class I and class II substances (1800 and 540 microgram/person/day, respectively). The total combined intake of candidate and supporting substances in Europe is approximately 4900 microgram/*capita*/day, which exceeds the thresholds of concern for structural class I and II (1800 and 540 microgram/person/day, respectively). However, for one of the supporting substances [FL-no: 02.056] a NOAEL exists, which provides an adequate margin of safety. Therefore, the total combined intake would not be expected to be of safety concern.

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude evaluating the candidate substances using the Procedure. All candidate substances, including the methacrylate esters, in this FGE are anticipated to be metabolised to innocuous products.

There are three methacrylate candidate substances in this group (ethyl methacrylate [FL-no: 09.375], methyl methacrylate [FL-no: 09.647] and isobutyl 2-methylprop-2-enoate [FL-no: 09.586]). It has been suggested in FGE.05 that these substances might cause neurotoxicity after oral exposure. However, after re-evaluation of the data available, the Panel concluded that the indications for this effect were not sufficiently underpinned. The alleged neurotoxicity was only reported at dose levels at least 7 orders of magnitude above the estimated levels of exposure based on the MSDI approach. Moreover, no indications for neurotoxicity were observed in a chronic oral study in rats with similar levels of exposure. Thus, the Panel concluded that there are no toxicity data which would preclude the evaluation of these three substances via the A-side of the Procedure-scheme.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure. It is considered that on the basis of the default MSDI approach all the 37 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

Uncertainties

In the EFSA opinion, uncertainties were not explicitly described. However, when looking at this case study it is clear from the Tier 0 approach that substantial uncertainties are attached to the tier 0 assumptions. These are:

- Uncertainties of production volumes (underreporting; although corrected)
- Occurrence and use levels
- Part of the population exposed (assumption 10%).
- Likelihood of co-exposure in reality

When needed more specific exposure calculations can be performed: i.e. Theoretical Added Maximum Daily Intake (mTAMDI).

On the hazard side, the assumption is made that based on structural similarities, common metabolites are formed exerting the same toxicity. Although this is very likely, the rate and likelihood of this process is not known.

Commercial Hexane Case Study

Tier 0 TTC Example for a Complex
Substance

Why Commercial Hexane?

- Constituents fairly well characterized
- Toxicology data for major constituents and on the complex substance
- Enables comparison of TTC estimates with data-based estimates to check utility of TTC approach as a conservative Tier 0 screen

Focus of Tier 0 Case Study

- Hazard – utilizes the TTC
- In Framework, would also consider refinement of exposure in higher Tiers if needed
- Exposure defined as worst case
 - For purposes of illustration, used point estimate for mixture based on a hypothetical population inhalation exposure, distributed across constituents by weight fractions
 - In practice, number of considerations
 - What is conservative , yet reasonable for Tier 0?
- Inhalation to oral route extrapolation to use TTCs
- Tier 0 TTC results - see Table 3
 - HI = 0.17

Comparison with Constituent Data

- DNELs based on REACH guidance
- Results in Table 4
 - HI = 0.0005
- IRIS inhalation RfCs each w/ UF=300, for 2 constituents yielded higher oral toxicity values than their corresponding TTC values
 - Would yield HI less conservative than TTC

Comparison with Complex Substance Data

- DNEL based on REACH guidance
 - Results in Table 5
 - HI values of 0.000013, 00012, 00071 based on substance composition (% of n-hexane)
 - HI's are more conservative than TTC
- Other complex substance benchmarks based on the C5-C8 hydrocarbon aliphatic fraction
 - HI = 0.001 (Total Petroleum Hydrocarbon Working Group) below TTC HI = 0.17
 - HI = 0.12 (Mass Department of Environmental Protection) Based on n-hexane data; virtually the same as TTC HI = 0.17

Uncertainties/Issues

- Exposure point estimate
- Fixed ratios of constituent proportions
- Route to route extrapolation to use TTCs with some portal of entry effects (nasal irritation)
- Broad range of toxicity values based on different methods of derivation
- Identified constituents all Cramer Class I with same TTC = 0.03
 - Based on fixed body weight and inhalation rate
 - Could be applied to entire complex substance based on structural similarity

Tier 0 Case Study – Commercial Hexane

[Text to illustrate the Powerpoint presentation]

Prepared by: The ILSI HESI Mixtures Project Committee

1. Introduction

The HESI Mixtures group has proposed the use of the Threshold of Toxicological Concern (TTC) approach as a Tier 0 assessment to prioritize the need for further evaluation of a chemical mixture. Based upon structural characteristics, substances can be grouped into classes (Cramer classes), each of which has an associated hazard benchmark denoted as a threshold of toxicological concern. Each TTC is designed to be a conservative estimate of hazard for a substance falling within its associated Cramer Class. This case study has been developed as a step in evaluating the performance of the TTC in a screening role. Commercial hexane, a complex hydrocarbon solvent, is an example of substances which contain multiple constituents because of the way in which they are manufactured. It was chosen for this case study because the constituents of commercial hexane are reasonably well characterized analytically and toxicological studies have been performed on commercial hexane as well as individual constituents accounting for the majority of its volume. Thus, the results of a screening evaluation utilizing the TTC can be compared both to the results of an evaluation obtained based upon data for the complex substance, as well as that of an additive approach based upon data for the individual constituents. For the TTC approach to be an effective conservative screen, it would be expected that a hazard index calculated using the TTC would exceed that of a hazard index calculated using data for the complex substance.

For component based approaches utilizing the TTC or hazard benchmarks for each constituent, dose addition is assumed, in which each component is assumed to contribute to the toxicity of the mixture in proportion to its weight percentage in the mixture.

This case study is a hypothetical example that utilizes available toxicological data as well as the TTC. It is not intended to be taken as an actual risk assessment for commercial hexane. A hypothetical exposure value is utilized for commercial hexane.

2. Considering the need for a framework analysis

What is the nature of exposure? Are the key components known? Are there data available on the hazard of the mixture itself?

Commercial hexane represents a complex substance. The principal constituent of this complex substance is n-hexane which has been associated with peripheral neuropathy in humans. Accordingly, the uses of this substance are believed to be restricted to specific industrial and professional applications in which exposures are controlled. However, for the purposes of this case study it is assumed that a low level air emission has occurred from an industrial facility during production or industrial use, leading to indirect exposure of the general population from the environmental release. Due to the physical chemical properties of commercial hexane, the most relevant route of general population exposure from an environmental release to air would be via inhalation. Thus, the scenario being examined will be a general population exposure via inhalation.

Key components are known, and there are data available both for the complex substance itself and the components present at the greatest weight fraction. For other components, toxicity can be estimated using relevant toxicological data for structurally similar substances.

Is exposure likely, taking into account the context?

For the purposes of this example, it is assumed that exposure to the general population is possible via inhalation of air.

Is there a likelihood of co-exposure within a relevant timeframe?

As all components are present in commercial hexane, co-exposure to all would be considered likely during a direct use of commercial hexane. Once released to the environment, constituents may undergo different fate and partitioning processes. For this hypothetical example, it is assumed all constituents would undergo similar environmental processes, and the mixture to which the general population is exposed would be present in the same ratios as in the liquid phase. Due to the similar physical and chemical properties of the constituents, this would seem a reasonable assumption.

What is the rationale for considering compounds in an assessment group?

All of the compounds are present in commercial hexane. All of the main constituents of commercial hexane also fall within the same Cramer Class.

3. Purpose and focus of the assessment

This case-study addresses a Tier 0 screening-level risk assessment for an assumed release of commercial hexane to air. It also compares results with higher tier data-based assessments utilizing two separate approaches: a) an additive approach based upon constituents and b) an approach based upon the entire composition. The purpose of this case study is to explore the WHO – IPCS combined exposure framework approach, and also the potential utility of the TTC as a Tier 0 screening tool within the WHO- IPCS framework. Three assessments will be performed: a Tier 0 additive constituent based approach using the TTC hazard benchmarks; a higher tier additive constituent based approach utilizing constituent toxicity estimated based upon constituent data or read-across to similar substances; and a higher tier estimate based upon toxicity data for the complex substance as a whole. These three assessments will assist in evaluating the use of the TTC as compared to actual data.

4. The framework analysis

4.1. Tier 0 - TTC

4.1.1. Exposure assessment

Commercial hexane is a complex and variable substance, but of fairly well characterized composition. The weight fractions of individual constituents present in a sample of commercial hexane are provided in Table 1 (Daughtrey et al., 1994). For the purposes of this example, a hypothetical worst case exposure to 0.005 mg/kg/day commercial hexane was assumed. The value is intended to represent an upper bound exposure based upon hypothetical measurements and representative of a highest exposed age group (i.e., the age group with the highest inhalation rate per body weight). Exposure values for individual constituents were estimated by multiplying the commercial hexane exposure value by the respective weight fraction of each constituent (found in Table 1).

The exposure values can be found in tables 3-5 under the risk evaluation.

Table 1. Constituents of liquid commercial hexane. Source: Daughtrey et al., 1994

Substance	% weight
n-hexane	52.2
3 methyl pentane	16
methyl cyclopentane	15.6
2-methyl pentane	11.6
cyclohexane	3.2
2,3, dimethyl butane	1.1
2,2 Dimethyl pentane	0.1
2,4 Dimethyl pentane	0.1
2,2 dimethyl butane	0.01
2,2,3 trimethyl butane	0.01
2 methyl hexane	0.01

4.1.2. Hazard assessment

This Tier 0 example assumes that no toxicological information, other than structure, is available for the detected substances. Cramer classification of each of the 10 substances was performed using ToxTree version 1.60 (<http://toxtree.sourceforge.net/>). Each constituent fell into Cramer Class 1. (Table 2).

Table 2. Cramer classes and associated individual TTC values

Substance	SMILES	Cramer Class	Cramer Value (ug/day)	Cramer Value (mg/kg/day)*
n-hexane	CCCCCC	I	1800	0.03
3 methyl pentane	CCC(C)CC	I	1800	0.03
methyl cyclopentane	C1(CCCC1)C	I	1800	0.03
2-methyl pentane	CC(C)CCC	I	1800	0.03
cyclohexane	C1CCCCC1	I	1800	0.03
2,3, dimethyl butane	CC(C)C(C)C	I	1800	0.03
2,2 Dimethyl pentane	CC(C)(C)CCC	I	1800	0.03
2,4 Dimethyl pentane	CC(C)CC(C)C	I	1800	0.03
2,2 dimethyl butane	CC(C)(C)CC	I	1800	0.03
2,2,3 trimethyl butane	CC(C)(C)C(C)C	I	1800	0.03
2 methyl hexane	CC(C)CCCC	I	1800	0.03
Using TTC and additivity, for mixture allowable level would be			1800	0.03

*(based upon 60 kg body weight used in Cramer Value derivation)

1.3. Risk characterization/analysis of uncertainties

For the constituent-based approaches, hazard quotients for each mixture component can be calculated by dividing the exposure values (as shown in table 1) by the respective hazard benchmark (TTC or DNEL) value:

$$HQ_{\text{individual substance}} = \frac{\text{Exposure}_{\text{individual substance}} (\text{mg/kg-bw/day})}{\text{Hazard benchmark}_{\text{individual substance}} (\text{mg/kg-bw/day})}$$

A Hazard Index (HI) for the mixture can be calculated by taking the sum of the individual hazard quotients. Assuming dose addition for all components, a higher tier assessment would not be a high priority if the Hazard Index <1.

$$HI_{\text{mixture}} = HQ_A + HQ_B + HQ_C + HQ_D \dots + HQ_J$$

Table 3: Tier 0 TTC Based Calculation of Hazard Quotients for each mixture component

Substance	% Weight	Hypothetical Exposure (mg/kg/day)*	Cramer Value (mg/kg/day)	Hazard Index
n-hexane	52.2	2.6E-03	0.03	8.7E-02
3 methyl pentane	16	8.0E-04	0.03	2.7E-02
methyl cyclopentane	15.6	7.8E-04	0.03	2.6E-02
2-methyl pentane	11.6	5.8E-04	0.03	1.9E-02
cyclohexane	3.2	1.6E-04	0.03	5.3E-03
2,3, dimethyl butane	1.1	5.5E-05	0.03	1.8E-03
2,2 dimethyl pentane	0.1	5.0E-06	0.03	1.7E-04
2,4 dimethyl pentane	0.1	5.0E-06	0.03	1.7E-04
2,2 dimethyl butane	0.01	5.0E-07	0.03	1.7E-05
2,2,3 trimethyl butane	0.01	5.0E-07	0.03	1.7E-05
2 methyl hexane	0.01	5.0E-07	0.03	1.7E-05
TOTAL				0.17

*Hypothetical mixture worst case exposure = 0.005 mg/kg bw/day

As shown in Table 3, the calculated hazard index of 0.17 is less than 1.0, and therefore the results of this Tier 0 assessment suggest that advancement to higher assessment tiers is not necessary in this example.

There are a number of uncertainties in this example, one of which is the TTC a suitable indicator to use at a Tier 0 level. This will be explored by comparing the results of the TTC approach to the higher tier estimates that follow. For the purposes of this case study, it is indicated that a worst case estimate of exposure is utilized. Uncertainties related to this would be how should a worst case estimate be defined for a screening tier, which may include consideration of: what is an appropriate upper bound percentile to utilize; temporal aspects such as appropriate exposure averaging time as compared to that of the selected hazard benchmark (i.e., monitored data may be for short periods as compared to chronic hazard benchmarks); and other assumptions that may enter into exposure estimates (for example, inhalation estimates for an age group with the highest inhalation rate per body weight basis are compared to a lifetime hazard benchmark).

It may be noted from this example that if all of the TTC values are in the same Cramer class, then the HI is mathematically the same when computed using the total mixture exposure divided by the Cramer Value for that class. In this case, we have $0.005/0.03 = 0.17$. This is interesting because if not all of the constituents of a mixture are known, but we get the same Cramer classification for a large number of constituents and/or percentage of the mixture, then it could be argued that the Cramer value could be applied to evaluate the whole mixture using its benchmark value. This then, begs the question of how stable the mixture composition is across exposure and time.

4.2 Higher Tier Constituent-Based Approach

While the Tier 0 assessment did not indicate the need for further evaluation ($HI < 1$), the purpose of going on to a higher tier analysis is to test the utility of the TTC as a Tier 0 approach.

4.2.1 Exposure Assessment – Higher Tier Constituent-Based Approach

The same exposure values as utilized in the Tier 0 approach are used for this higher tier assessment. The values are intended to be conservative and reflect hypothetical monitoring data. As the Tier 0 assessment did not indicate the need for further evaluation, there was no need to refine the exposure portion.

4.2.2 Hazard Assessment - Higher Tier Constituent-Based Approach

For individual components, relevant hazard benchmarks developed in compliance with the Registration, Evaluation and Authorization of Chemicals (REACH) regulation methodology were utilized when available. These hazard benchmarks were derived based upon substance and constituent-specific toxicology data and regulatory advice and followed REACH guidelines for Derived No Effect Level (DNEL) development for the general population, and are designed to be health protective for chronic lifetime exposures. For some components which did not have specific toxicology data, DNELs were assumed to be equivalent to those derived from the most relevant data set. For these components, the whole-substance (commercial hexane) data was generally utilized. Constituent hazard benchmarks are provided in Table 4. Details of DNEL derivation are provided in Appendix A.

Table 4: Higher Tier Constituent Based Calculation of Hazard Quotients for each mixture component

Substance	% Weight	Hypothetical Exposure (mg/kg/day)	Hazard Benchmark (DNEL) (mg/kg/day)	Hazard Index
n-hexane	52.2	2.6E-03	5	5.2E-04
3 methyl pentane	16	8.0E-04	377	2.1E-06
methyl cyclopentane	15.6	7.8E-04	377	2.1E-06
2-methyl pentane	11.6	5.8E-04	377	1.5E-06
cyclohexane	3.2	1.6E-04	69	2.3E-06
2,3, dimethyl butane	1.1	5.5E-05	377	1.5E-07
2,2 dimethyl pentane	0.1	5.0E-06	149	3.4E-08
2,4 dimethyl pentane	0.1	5.0E-06	149	3.4E-08
2,2 dimethyl butane	0.01	5.0E-07	377	1.3E-09
2,2,3 trimethyl butane	0.01	5.0E-07	149	3.4E-09
2 methyl hexane	0.01	5.0E-07	149	3.4E-09
TOTAL		0.005		0.0005

4.2.3 Risk Characterization / Uncertainties - Higher Tier Constituent-Based Approach

In this approach, the same constituent-specific exposure values are utilized as in the TTC approach, but the hazard benchmarks represent data-derived DNELs. Results are provided in Table 4. The hazard quotient is 0.0005, so lower than 1 and lower than the estimated HQ of the TTC-based approach by 4 orders of magnitude. The DNEL derivation approach is designed to be conservative, and the hypothetical exposure was also defined as a conservative value. Further discussion of uncertainties and alternate hazard benchmarks will be addressed in section 5.

4.3 Higher Tier Mixture-Based Approach

Commercial hexane was chosen as an interesting case study because data are available for the complex substance itself as well as its primary constituents. In this section, whole substance data are utilized in DNEL derivation.

4.3.1 Exposure Assessment – Higher Tier Mixture-Based Approach

The same exposure values as utilized in the Tier 0 approach are used for this higher tier assessment. The values are intended to be conservative and reflect hypothetical monitoring data. As the Tier 0 assessment did not indicate the need for further evaluation, there was no need to refine the exposure portion.

4.3.2 Hazard Assessment - Higher Tier Mixture-Based Approach

In this approach, the hazard benchmark for the entire complex substance is developed and presented in two ways, both using REACH guidance for DNEL derivation: a) based upon the toxicology study results for the complex substance alone and b) in a conservative approach used for REACH submissions, in which the complex substances (at 52% n-hexane) data-derived DNEL was used for constituents other than n-hexane and for n-hexane, which has unique toxicological properties, the DNEL was based upon the regulatory control value (i.e., the European Indicative Occupational Exposure Limit Value) with appropriate adjustment factors to derive a general population DNEL. The REACH hydrocarbons, C6, n-alkanes, isoalkanes, cyclic, n-hexane rich DNEL is derived to cover substances including commercial hexane in which the complex substance may range in n-hexane content from 20 – 80%. Therefore, for REACH DNEL derivation, to be conservative, an 80% concentration of n-hexane was assumed as well as considering data for commercial hexane (at 52% n-hexane) for the remaining portion of the complex substance. For the purposes of this case study, hazard benchmarks and hazard quotients are also calculated assuming a 52% concentration of n-hexane (to be consistent with the n-hexane weight fraction in the constituent approach) and using the data for commercial hexane alone (Table 5).

4.3.3 Risk Characterization - Higher Tier Constituent-Based Approach

Risk characterizations based upon the 3 DNEL approaches described are presented in Table 5.

Table 5: Higher Tier Mixture-Based Calculation of Hazard Quotient

Commercial Hexane: approach used for benchmark development	Hypothetical Exposure (mg/kg/day)	Hazard Benchmark (DNEL) (mg/kg/day)	Hazard Index
Whole Complex Substance: data for commercial hexane (which is ~52% n-hexane)	0.005	377	1.3E-05
Est. for case study: whole complex substance (which is ~52% n-hexane) data-derived DNEL weighted at 48%, and n-hexane data-derived DNEL weighted at 52%	0.005	42	1.2E-04
REACH DNEL: whole complex substance (which is ~52% n-hexane) data-derived DNEL weighted at 20%, n-hexane data-derived DNEL weighted at 80%	0.005	7	7.1E-04

These values are well below one, and similar to or lower than the data-derived constituent-additive approach (a portion of which was based upon the complex substance data) and orders of magnitude below the TTC approach (HI = 0.17).

5. Risk Characterization / Uncertainties - General Discussion

5.1 Alternate hazard benchmarks

For the purposes of this example, REACH DNELs were used because they were derived based upon regulatory guidance and available for all constituents as well as the whole complex substance. However, alternate hazard benchmarks could also be assessed. For example IRIS values are available for 2 of the constituents, n-hexane and cyclohexane: inhalation RfCs are 0.7 mg/m³ and 6 mg/m³ respectively. Utilizing common default values of 20 m³ inhalation/day and 60 kg body weight, these values convert to 0.2 mg/kg/day for n-hexane and 1.7 mg/kg/day for cyclohexane. Both values include a 300-fold uncertainty factor in their derivation, and are 1-2 orders of magnitude greater than the TTC values for these compounds, consistent with the TTC being a conservative screen. Their use would result in a hazard index falling below that calculated using the TTC (i.e., would indicate less potential for toxicity).

Additional alternative values for comparison, include hazard benchmarks for the EC5-EC8 Aliphatic Fraction of total petroleum hydrocarbons that can be used as surrogates for commercial hexane hazard. The Total Petroleum Hydrocarbon Working Group (TPHCWG) and the Massachusetts Department of Environmental Protection (MADEP) have set hazard benchmark values of 5.0 and 0.04 mg/kg-day for this fraction (MADEP, 2003), resulting in HIs of 0.001 and 0.12, respectively. The MADEP values are based upon n-hexane. These HIs are also below the TTC approach HI, with the TPHCWG HI three orders of magnitude below and the MADEP value being essentially equivalent. These comparisons provide further evidence of conservatism using the TTC approach.

5.2 Additional Uncertainties

This is a hypothetical example, and there are many uncertainties in it. The information was provided only as an example in a hypothetical situation and was not intended to be a formal risk assessment for commercial hexane. Several points to consider include that TTCs are based upon oral intake, and the hazard benchmarks utilized were based upon inhalation exposure. This is particularly problematic for compounds where portal of entry effects have been observed (e.g., nasal irritation or lung lesions). For commercial hexane, although some effects in the nasal tissues have been observed, neurotoxicity for n-hexane appears to be the primary effect of concern (as described in MADEP, 2003). Oral RfDs were not available in IRIS for these constituents. Oral REACH DNELs were 6 mg/kg/day for n-hexane as compared to the inhalation values utilized (Appendix A). The exposure value is a hypothetical one and is described as representing a worst case (high end) exposure. Aspects related to what is an appropriate high end exposure to compare to a chronic hazard benchmark should be considered. Conversion of hazard benchmarks in mg/m³ (IRIS values) to mg/kg/day was done based upon adult inhalation and body weight defaults.

A useful aspect of this analysis was that data-derived hazard benchmarks for commercial hexane and its constituents were all derived using common methodology (i.e., REACH DNEL guidance).

6. Conclusions

This hypothetical case study demonstrates the potential utility of applying the TTC approach as a Tier 0 assessment tool for chemical co-exposures. All 3 approaches [TTC, constituent additivity, and data for the complex substance] resulted in hazard quotients of < 1, indicating no need for further assessment as the hypothetical exposure fell below the hazard benchmarks. The results, in order of decreasing hazard index, were: TTC (0.17), Fraction-based MADEP (0.12) and TPHCWG (0.001), Constituent-based data-derived DNEL: (0.0005), complex substance-based data derived DNEL (0.000015). Thus, for this case study the TTC-based value provided a conservative risk estimate as compared to either the whole substance or the constituent additivity approaches.

7. References

Daughtrey, W.C., T. Neeper-Bradley, J. Duffy et al. 1994. Two-generation reproduction study on commercial hexane solvent. *J. Appl. Toxicol.* 14:387-393.

MADEP (Massachusetts Department of Environmental Protection). 2003. Updated petroleum hydrocarbon fraction toxicity values for the VPH/EPH/APH methodology. Office of Research and Standards, Massachusetts Department of Environmental Protection, Boston, MA.

Appendix A. Derivation of REACH DNEL

Table A1. Summary of commercial hexane constituents, relevant dossiers and DNEL basis

General Population Long Term Systemic Effect DNELs

Substance	REACH Dossier	Basis	Inhalation (mg/m3)	Inhalation DNEL in mg/kg/day*	Dermal (mg/kg/day)	Oral (mg/kg/day)
Commercial hexane (assume 80% n-hexane)	Hydrocarbons, C6, n-alkanes, isoalkanes, cyclic, n-hexane rich	Data for commercial hexane at 52% n-hexane (0.2 weight) and IOEL for n-hexane (0.8 weight) adjusted for general population**	20	7	7	6
For case study - commercial hexane - utilize 52% n-hexane and 48% commercial hexane		Data for commercial hexane at 52% n-hexane (0.48 weight) and IOEL for n-hexane (0.52 fraction) adjusted for general population**	125	42		
Commercial hexane (as tested, which includes ~52% n-hexane)	Hydrocarbons, C6, n-alkanes, isoalkanes, cyclic, n-hexane rich	Data for commercial hexane at 52% n-hexane concentration	1131	377	1377	1301
n-hexane	See discussion in Hydrocarbons, C6, n-alkanes, isoalkanes, cyclic, n-hexane rich for basis for n-hexane alone	Worker inhalation DNEL for n-hexane = Indicative Occupational Exposure Level (IOEL) = 75 mg/m3; for general population conversion values as discussed in the cited document**	16	5		
3 methyl pentane	C6 isoalkanes < 5% n-hexane	Data for commercial hexane at 52% n-hexane concentration	1131	377	1377	1301
methyl cyclopentane	C6 isoalkanes < 5% n-hexane	Data for commercial hexane at 52% n-hexane concentration	1131	377	1377	1301
2-methyl pentane	C6 isoalkanes < 5% n-hexane	Data for commercial hexane at 52% n-hexane concentration	1131	377	1377	1301
cyclohexane	Cyclohexane	Data for cyclohexane; for inhalation general population extrapolated from IOEL = SCOEL values	206	69	1186	59.4
2,3, dimethyl butane	C6 isoalkanes < 5% n-hexane	Data for commercial hexane at 52% n-hexane concentration	1131	377	1377	1301
2,2 dimethyl pentane	Hydrocarbons, C7, n-alkanes, isoalkanes, cyclics	Data for n-heptane	447	149	149	149
2,4 dimethyl pentane	Hydrocarbons, C7, n-alkanes, isoalkanes, cyclics	Data for n-heptane	447	149	149	149
2,2 dimethyl butane	c6 isoalkanes < 5% n-hexane	Data for commercial hexane at 52% n-hexane concentration	1131	377	1377	1301
2,2,3 trimethyl butane	Hydrocarbons, C7, n-alkanes, isoalkanes, cyclics	Data for n-heptane	447	149	149	149
2 methyl hexane	Hydrocarbons, C7, n-alkanes, isoalkanes, cyclics	Data for n-heptane	447	149	149	149

*20 m3/day and 60 kg/day

**10/20 to correct for difference between worker and general population ventilation rates, 5/7 to correct for days of the week to which the general population could be exposed, and 3/5 to utilise the assessment factor of 5 for the intraspecies differences)

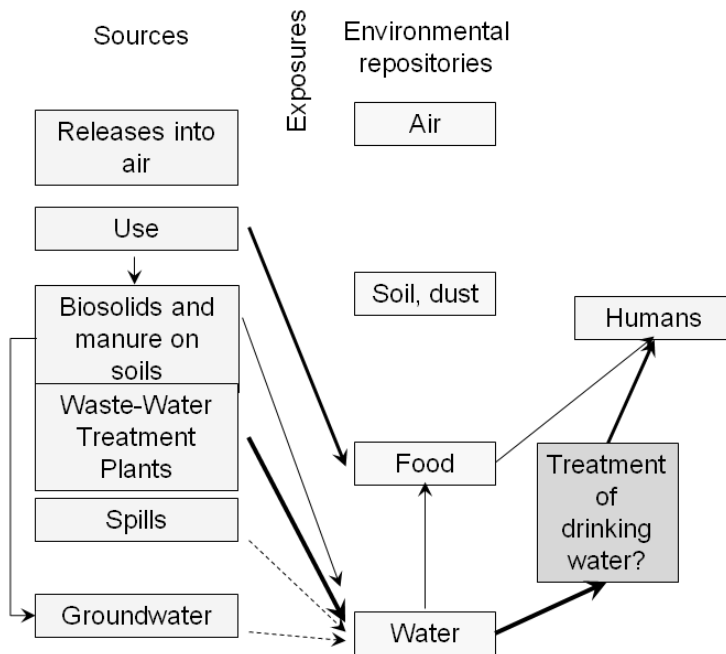
HESI Pharm TTC Case study

Keith Solomon et al.

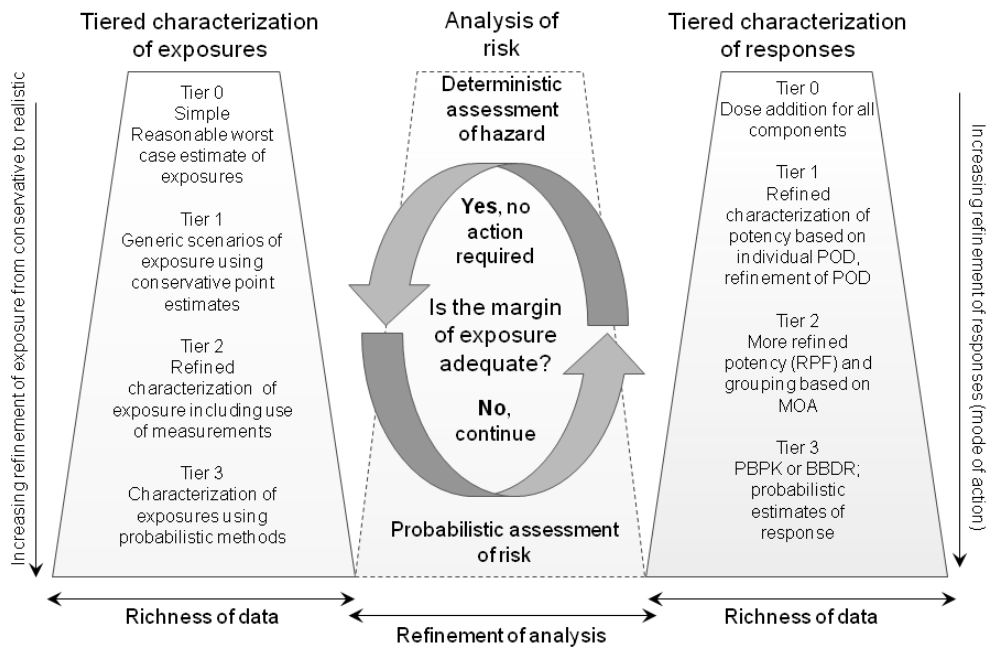
Pharmaceuticals in surface waters

- Recently found in surface waters – new analytical procedures.
- Biologically active compounds for which therapeutic and toxicity values are known.
- Tiered approach.

Conceptual model of exposures



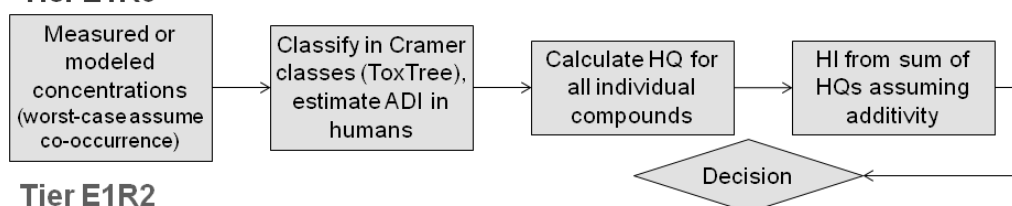
Tiered framework



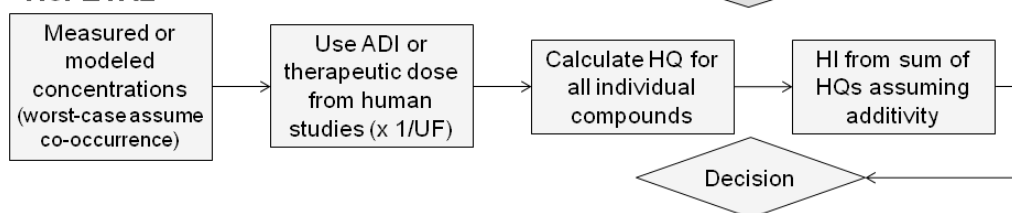
Proof of principle

- 94 pharmaceuticals classified by Cramer Class using ToxTree (2009).
 - 5 compounds in Cramer Class 1 or Class 2
 - All others were in Cramer Class 3.
 - Kroes evaluation using the ToxTree indicated that 17 Cramer Class 3 compounds would require individual evaluations or be limited to exposure of 0.15 µg/d.
- When compared to published the 75 ADIs available, the TTC evaluation (even with the Kroes exposure restriction of 0.15 µg/d) was:
 - Too high for 35% of the comparisons, and too low in 65%
 - TTC approach not conservative enough to use as an initial tier in the assessment of mixtures of pharmaceuticals.

Tier E1R0



Tier E1R2



Tier E1RO

- Exposure concentrations
 - Worst case
 - Maximum concentrations reported in surface water
 - No removal by treatment
- TTC calculated and,
- Therapeutic dose used to determine TQ
 - UF of 1000 and 10,000

$$TQ = \frac{\text{Exposure value (mg/kg/day)}}{\text{Minimum therapeutic value (mg/kg/day)} \div UF}$$

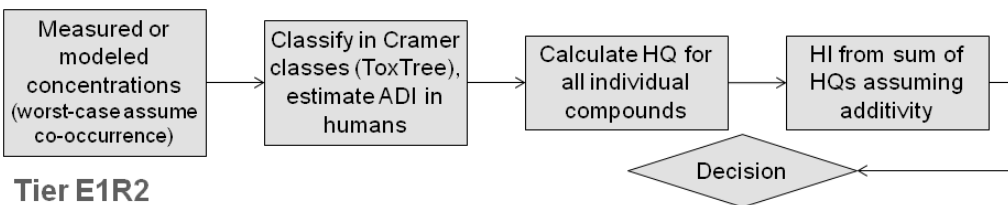
Tier E0R1 and E1R2 results (Case 2)

- Cramer classification and TTC
 - HI = 0.84 – low risk from worst-case combined
- TQ approach
 - UFs of 10,000 – HI= 17.7
 - UFs of 1000 – HI = 1.8
- The TTC approach might not be as conservative as the use of ADIs.
- HI values were close to or > 1, but extreme worst-case assumptions were made – greatest reported concentration and additivity across all mechanisms of action.

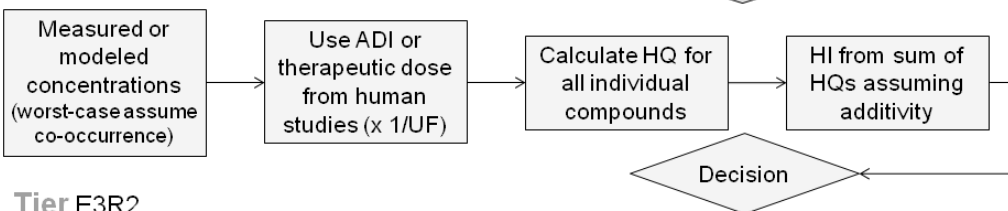
Tier E1R2 (Case 3)

- Measured concentrations of pharmaceuticals from treated drinking water in several locations in the US.
 - Worst-case - greatest reported concentration
 - Drinking Water Exposure Level (DWEL) from Bruce et al. (2010) used to calculate HQs and a HI.
- HI = 0.0175.
 - Assuming worst-case additivity across classes and co-occurrence of the highest reported concentrations, risks from mixtures of pharmaceuticals measured in treated drinking water were below the level of concern. (Conclusion - treatment reduces exposures)

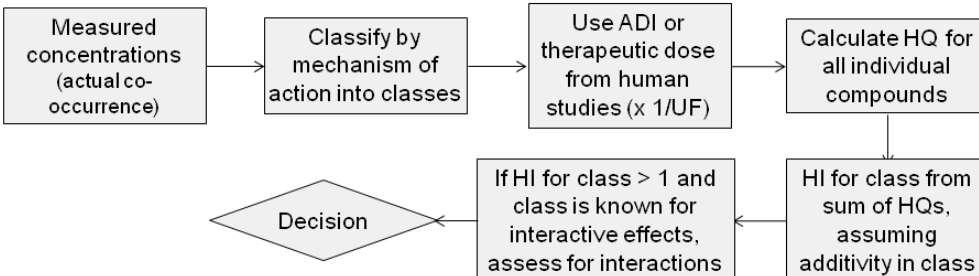
Tier E1R0



Tier E1R2



Tier E3R2



Tier E3R2 (Case 4)

- HI by class. Estimated exposures to estrone, 17- β estradiol, estriol, and 17- α ethinyl estradiol (Caldwell et al. 2010).
- HIs using TTC limits, Australian guideline surrogate sADIs (EPHC 2008), and ADIs determined by product manufacturers for workplace exposures.
- HIs
 - TTC = 7.94×10^{-7}
 - Australian sADIs = 0.0392
 - Workplace ADIs = 0.0214
- TTC significantly underestimates risk

Tier E3R2 (Case 5)

- HI by class. Antibiotics from Case 2
- HQs based in $TD \div UF$
- HI
 - UF of 1000 = 5.70×10^{-3}

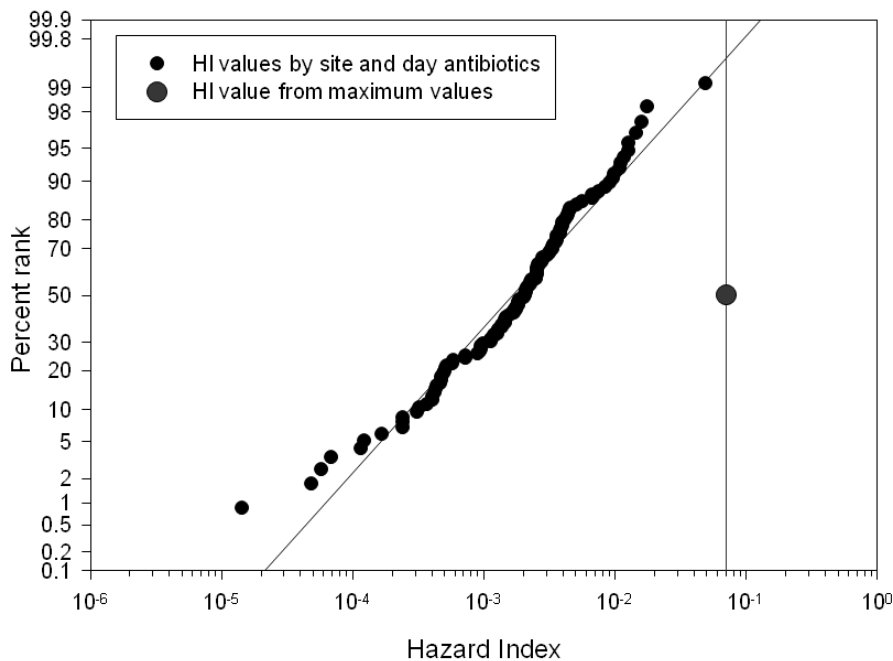
Tier E3R2 (Case 6 not in report)

- Data on antibiotics (and carbamazepine) from Lissemore et al. (2006) on surface water (Grand River, Ontario).
- Samples 2-weeks apart.
- Chemicals analyzed:
 - Monensin, lincomycin, trimethoprim, sulfadimethoxine, sulfamethazine, total erythromycin, sulfathiazole, sulfamethoxazole, doxycycline, sulfachlorpyridazine, sulfamerazine, sulfamethizole, roxithromycin, oxytetracycline, tylosin, tetracycline, chlortetracycline

Case 6 analysis

- HQs from time- and location-specific measurements and Australian sADIs.
- HIs calculated for each day and location.
- Plotted probabilistically and compared to worst case (maximum for all days and sites).

Case 6 results



Overall conclusions 1

- Use of a TTC does not appear to always provide the most conservative screening approach for pharmaceuticals.
- Use of the Australian guidelines appears to provide a conservative screen for determining surrogate ADIs for pharmaceuticals and is based on readily-available data on therapeutic doses.
- Given that there is generally more data on the biological activity of pharmaceuticals, the TTC approach may be inappropriate anyway as higher tiers can be just as readily applied.
- Adding HIs within therapeutic classes provides a conservative evaluation of pharmaceutical mixtures.

Assumptions

● Hazard

- Large UFs on TD may be too conservative

● Exposure

- Worst case gives upper bound, if HI is <1 , no problem.

● Interactions

- Only when HI >1 but this is tempered by the UFs.

Tier 0 Case Study – Pharmaceuticals potentially detectable in surface water

[Text to illustrate the Powerpoint presentation]

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INTRODUCTION

It was only recently that pharmaceuticals and personal care products were identified as potential “chemicals of concern” in surface water (Daughton and Ternes 1999, Kolpin et al. 2002, Lissemore et al. 2006, ter Laak et al. 2010, Brausch and Rand 2010). This was primarily due to the increased availability of methods of analysis based on LC-MS which allow for the detection of relatively polar molecules, such as the majority of pharmaceuticals, at very low concentrations.

Unlike most other chemicals, pharmaceuticals and substances used for veterinary purposes normally have significant databases designed to evaluate possible effects in humans and animals from intended and expected exposures. Even so, many of these individual products do not have officially determined acceptable daily intake (ADI) values. Screening the possible hazard from exposure to complex mixtures of these substances at very low concentrations could be quite time consuming if regular practices for deriving ADI values were used. Yet proposed methods of screening using TTC values might not capture the hazard properties associated with pharmaceuticals designed to affect specific therapeutic targets with, in some cases, high potency.

The intent of this case study was to illustrate the potential utility of applying various approaches in a Tier 0 to Tier 1 assessment to prioritize the need for further evaluation of a mixture of pharmaceutical residues. This case study uses the Cramer et al. (1978) classes, which have been used to define three potency bins for non-cancer endpoints in the TTC approach. The approach assumes that the only information available is 1) the identities and structures of the chemicals in the mixture and 2) their respective concentrations in surface water. Based on these two sets of data, the TTC approach (Kroes et al. 2004) was evaluated as a Tier 0 assessment tool. Furthermore, additional evaluations of mixtures of pharmaceutical were conducted using a variety of approaches to identify at least one appropriately conservative screening technique.

The first case study was a direct evaluation of distribution of TTC classes for a sample of 94 pharmaceuticals based only on the structures of the chemicals alone. The TTC values were then compared to published ADI values available for 75 of the pharmaceuticals to determine how conservatively the TTC values predicted actual ADI values.

Additional case studies were conducted to evaluate pharmaceutical mixtures. The studies used a Hazard Index (HI) derived from the sum of the Hazard Quotients (HQ) to characterize the hazard of the mixture. The second case-study estimated HQs from a data set of maximum measured concentrations of pharmaceuticals in surface water for each compound and the TTC for the same compound. The third evaluation derived the HI from the same data set of maximum measured concentrations in surface water and from the lowest therapeutic dose divided by an Uncertainty Factor (UF). Maximum measured concentrations from actual drinking water samples and calculated Drinking Water Exposure Levels were used to determine a HI in a fourth evaluation. Finally, HIs were derived for two therapeutic class groupings of pharmaceuticals. Near maximum drinking water concentrations of estrogen agonists were modeled and used to calculate Hazard Quotients with TTC values, surrogate ADIs using Australian

⁴ The author wishes to thank the members of the HESI Mixtures Panel for their useful input, comments, and suggestions.

guidelines, or calculated ADI values using workplace exposure guidelines (Caldwell et al. 2010). Hazard Quotients were also derived for a group of antibiotics from maximum surface water concentrations and surrogate ADIs using the Australian guidelines.

All of the examples assumed dose addition, where all components are considered to contribute to the toxicity of the mixture. This approach does not explicitly address the possibility of synergy. As discussed in the WHO/IPCS framework document, the use of dose additivity is considered conservative based on analysis of empirical results for effects of combined exposure including to chemicals that induce critical effects by different modes of action (USEPA 2007, EFSA 2008, European Commission 2010). In addition, in the small number of cases where it has been reported that dose additivity may under-predict effects as a result of synergistic interaction, recent analyses of limited available data suggest that the magnitude of the under-prediction is less than an order of magnitude (Kortenkamp and Hass 2009, European Commission 2010).

In a recent exercise, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) performed a literature review focused on findings of synergy at or near doses associated with a study's points of departure (e.g., the benchmark dose [BMD], no-observed-effect level [NOEL], etc.) that may be associated with the development of chronic health-based guidance values of individual mixture components. This review found few studies that reported a quantified value for synergy at the low doses used; from those studies, the maximum potential magnitude of synergy did not exceed approximately fourfold (e.g., less than four times that predicted based on an assumption of dose addition). However, most of the studies included in the review were performed at doses that caused overt toxic effects from acute exposures (Embry et al. 2009, Boobis et al. 2011). The additive model is expected to exclude exposures to such levels. Although the examples identified in this review were not sufficient to support robust conclusions regarding the frequency with which synergy is likely to occur following low-dose chronic exposures, these results might indicate an upper-bound estimate when screening of untested mixtures for synergistic potential. It has yet to be determined how such information should inform a Tier 0 risk assessment.

ANALYSIS OF PHARMACEUTICAL MIXTURES USING TTC VALUES AND ADI VALUES

Exposure and Hazard assessment

Exposure assessment

Concentrations of compounds used to derive Hazard Quotients are explained in the presentation for each example.

Hazard assessment

Cramer classification of each of compound was performed using ToxTree (2009). For purposes of calculating Hazard Quotients, the assumption was made that none of the compounds was genotoxic or excluded from the TTC approach (e.g., aflatoxin-like-, azoxy- and N-nitroso- compounds, polyhalogenated dibenzo-*p*-dioxins and dibenzofurans, steroids, proteins, high molecular weight chemicals such as polymers and nonessential metals).

The TTC approach relies on conservative daily exposure thresholds for each Cramer class, derived from a database of subchronic, chronic and reproductive/developmental oral toxicity data on more than 600 chemicals (Munro et al. 1996). The most conservative NOEL values were plotted based on structural class, and values corresponding to the 5th centile NOEL were selected, multiplied by 60 kg (average adult body weight) and divided by an uncertainty factor of 100 to calculate the human exposure threshold (i.e., TTC) values of 1800 µg/day (Cramer class I), 540 µg/day (Cramer class II) and 90 µg/day (Cramer class III). To use these TTC values for comparison with exposures of populations other than adults, the values should be converted back into units of milligrams per kilogram of body weight per day. Accordingly, Cramer class I chemicals have human exposure threshold values of 0.03 mg/kg body weight per day; class II, 0.009 mg/kg body weight per day; and class III, 0.0015 mg/kg body weight per day (Barlow 2005).

Risk characterization

Hazard quotients (HQ) for each mixture component was calculated by dividing the exposure values by the respective TTC or ADI value:

$$HQ_{\text{individual substance}} = \frac{\text{Exposure}_{\text{individual substance}} (\text{mg/kg bw/d})}{\text{Toxicity value or ADI}_{\text{individual substance}} (\text{mg/kg bw/d})}$$

A hazard index (HI) for the mixture can be calculated, assuming dose addition for all components, by taking the sum of the individual hazard quotients:

$$HI_{\text{mixture}} = HQ_A + HQ_B + HQ_C + HQ_D \dots + HQ_J$$

Higher-tier assessment would not be a high priority if the hazard index is less than 1. Utilizing the hazard index in this way corrects exposure values based on estimates of chemical potency.

Tier 0 – Tier 1 evaluations**Study one**

As an initial step to evaluate the TTC approach, 94 pharmaceuticals were classified by Cramer Class using ToxTree(2009). This process yielded only five compounds in Cramer Class 1 or Class 2 (Table 1). All of the rest of the compounds were in Cramer Class III. Kroes evaluation using the ToxTree model identified compounds that would normally be excluded from the Cramer classification process based on similarity to characterized compounds that have a hazard profile more potent than those included in Cramer classifications. Evaluation with the Kroes criteria indicated that 17 Cramer Class III compounds would require individual evaluations or be limited to exposure of 0.15 µg/d. When compared to published 75 ADI values that were available (Bruce et al. 2010, Cunningham et al. 2009, Schwab et al. 2005, Bercu et al. 2010), the TTC evaluation (even with the Kroes exposure restriction of 0.15 µg/d) was too high for 35 percent of the comparisons (Table 2). From this, we concluded that the TTC approach based on Cramer classes was not conservative enough to use as an initial tier in the assessment of mixtures of pharmaceuticals.

Table 1. Results of the screening exercise using TTC and Cramer Classes

Number of pharmaceuticals/ veterinary products evaluated for Cramer Class and for Kroes restrictions via ToxTree	Percentage restricted by Kroes analysis to individual evaluations or total daily exposure of 1.5 µg/kg/d	Percentage in Cramer Class III (Total daily exposure limit of 90 µg/d)	Percentage in Cramer Class II (total daily exposure limit of 540 µg/d)	Percentage in Cramer Class I (total daily exposure limit of 1800 µg/d)
94	18	77	3	2

Table 2. TTC screening levels compared to published ADIs

Number of compounds with published ADIs In screening evaluation	Percentage of compounds with ADIs ≤ TTC value	Percentage of compounds with ADIs > TTC value
75	35	65

Key Points

- The TTC approach can underestimate the ADIs for this group of biologically active compounds and may not be conservative enough to use as a screening approach.

Study two

Measured concentrations of pharmaceuticals in surface waters were obtained from the published literature and reports available to the public. For the purpose of this characterization, the data for concentrations in surface waters and toxicity were analyzed by using the greatest measured concentration and then calculating a therapeutic quotient (TQ) with an UF, as discussed below. Assuming the absence of data on ADI, TTC values were calculated as described above and are included in Table 3 for comparison purposes. As data were available, therapeutic doses in humans were used to assess hazards to humans via a therapeutic quotient (TQ). It was assumed that humans would be exposed to the concentrations measured in surface waters and that treatment of drinking water would not remove any compound. Because treatment of drinking water does remove some, if not all pharmaceuticals, risks were therefore overestimated and represent a worst-case. For all estimates of exposures, it was assumed that an average adult weighs 70 kg and consumes 2 L of water per day. The highest reported concentration in surface water was used in the calculation of the quotient was converted from ng/L to a dose in mg/kg/day. An UF of 1000 or 10,000 was applied to the calculation of the TQ.

$$TQ = \frac{\text{Exposure value (mg/kg/day)}}{\text{Minimum therapeutic value (mg/kg/day)} \div UF}$$

As outlined in Price et al. (2009) the hazard index (HI) was used to sum the hazards from all of the pharmaceuticals for which data were collected. In doing this, it is assumed that all the maximum concentrations were present in the same location at the same time – a highly unlikely worst-case event but suitable for low-tier analysis. In addition, it was assumed that the toxicity was additive across all cases of mechanism of action.

Use of Cramer classification and the TTC approach to calculate the HI gave a value of 0.84 (Table 3), suggesting that risk from worst-case combined exposures was well below a threshold of concern as based on TTC. However, when UFs of 10,000 and 1000 were applied to the TQs, the HI exceeded 1 (17.7 and 1.8, respectively, Table 3). Use of the uncertainty factors on the TQ gave a total HI somewhat higher than that from the TTC approach. While one can argue about the UF used here, they are not out of line from those used by others (Cunningham et al. 2009, EPHC 2008), these results confirm that the TTC approach could underestimate the risk for pharmaceuticals and their mixtures.

Key Points

- The TTC approach might not be as conservative as the use of ADIs.
- HI values were close to or > 1, but extreme worst-case assumptions were made – greatest reported concentration and additivity across all mechanisms of action.

Table 3. Hazard quotients for adults for consumption of pharmaceuticals in drinking water calculated from worst-case global data using Cramer Classes, and UFs on the therapeutic dose

Product	Max. concentration in surface water (ng/L)	Cramer Class	TTC value (mg/kg bw/ day)	Oral dose in adult (mg/kg bw/day)	TTC Hazard Quotient	Min. Adult therapeutic dose (mg/kg bw/d) UF=10,000	MEC/therapeutic dose	
							UF=10,000	UF=1,000
Amoxicillin	11	3	0.0015	3.1E-07	2.1E-04	1.1E-03	2.9E-04	2.9E-05
Cephalexin	26.8	3	0.0015	7.7E-07	5.1E-04	1.4E-03	5.4E-04	5.4E-05
Roxithromycin	180	3	0.0015	5.1E-06	3.4E-03	4.3E-04	1.2E-02	1.2E-03
Sulfamethoxazole	1340	3	0.0015	7.0E-06	4.6E-03	1.6E-02	4.4E-04	4.4E-05
Ciprofloxacin	0.182	3	0.0015	5.2E-09	3.5E-06	7.1E-04	7.3E-06	7.3E-07
Chlor-tetracycline	690	3	0.0015	4.7E-06	3.1E-03	1.4E-03	3.3E-03	3.3E-04

Product	Max. concentration in surface water (ng/L)	Cramer Class	TTC value (mg/kg bw/ day)	Oral dose in adult (mg/kg bw/day)	TTC Hazard Quotient	Min. Adult therapeutic dose (mg/kg bw/d) UF=10,000	MEC/therapeutic dose	
							UF=10,000	UF=1,000
Oxy-tetracycline	47000	3	0.0015	1.3E-05	9.0E-03	3.3E-04	4.0E-02	4.0E-03
Fluorouracil	27	3	0.0015	7.7E-07	5.1E-04	4.6E-02	1.7E-05	1.7E-06
Cyclo-phosphamide	146	3	0.0015	4.2E-06	2.8E-03	1.0E-04	4.2E-02	4.2E-03
Fluoxetine	99	3	0.0015	2.8E-06	1.9E-03	2.9E-05	9.9E-02	9.9E-03
Paroxetine	11.7	3	0.0015	3.3E-07	2.2E-04	2.8E-05	1.2E-02	1.2E-03
Metformin	130	3	0.0015	3.7E-06	2.5E-03	2.9E-03	1.3E-03	1.3E-04
Ivermectin	88	3	0.0015	2.5E-06	1.7E-03	2.0E-05	1.3E-01	1.3E-02
Thiabendazole	6	3	0.0015	1.7E-07	1.1E-04	5.0E-03	3.4E-05	3.4E-06
Carbamazepine	344	3	0.0015	9.8E-06	6.6E-03	5.7E-04	1.7E-02	1.7E-03
Oseltamivir	3	3	0.0015	8.6E-08	5.7E-05	2.4E-04	3.6E-04	3.6E-05
Digoxin	130	3	0.0015	3.7E-06	2.5E-03	1.3E-05	3.0E-01	3.0E-02
Atenolol	1200	3	0.0015	3.4E-05	2.3E-02	3.6E-05	9.5E-01	9.5E-02
Propranolol	120	3	0.0015	3.4E-06	2.3E-03	3.0E-03	1.1E-03	1.1E-04
Enalapril	150	3	0.0015	4.3E-06	2.9E-03	1.0E-05	4.3E-01	4.3E-02
Gemfibrozil	4760	2	0.09	1.4E-04	1.5E-03	2.0E-03	6.8E-02	6.8E-03
Clofibrate	4000	3	0.0015	1.1E-04	7.6E-02	2.0E-03	5.7E-02	5.7E-03
Diltiazem	49	3	0.0015	1.4E-06	9.3E-04	1.2E-02	1.2E-04	1.2E-05
Cimetidine	580	3	0.0015	1.7E-05	1.1E-02	5.7E-04	2.9E-02	2.9E-03
Omeprazole	8690	3	0.0015	2.5E-04	1.7E-01	2.9E-05	8.6E+00	8.6E-01
Ranitidine	2000	3	0.0015	5.7E-05	3.8E-02	1.1E-04	5.2E-01	5.2E-02
Testosterone	214	3	0.0015	6.1E-06	4.1E-03	1.0E-03	6.1E-03	6.1E-04
17 α -ethynyl-estradiol	0.9	2	0.09	2.6E-08	2.9E-07	1.0E-08	2.6E+00	2.6E-01
Estradiol	44	2	0.09	1.3E-06	1.4E-05	2.5E-05	5.0E-02	5.0E-03
Norethindrone	872	3	0.0015	2.5E-05	1.7E-02	1.0E-05	2.5E+00	2.5E-01
Acetyl-salicylic acid	600	1	0.03	1.7E-05	5.7E-04	5.0E-03	3.4E-03	3.4E-04
Acetaminophen	10000	3	0.0015	2.9E-04	1.9E-01	1.3E-01	2.2E-03	2.2E-04
Ibuprofen	16500	1	0.03	4.7E-04	1.6E-02	2.0E-02	2.4E-02	2.4E-03
Naproxen	5220	3	0.0015	1.5E-04	9.9E-02	1.5E-03	9.9E-02	9.9E-03
Diclofenac	930	3	0.0015	2.7E-05	1.8E-02	1.0E-02	2.7E-03	2.7E-04
Codeine	320	3	0.0015	9.1E-06	6.1E-03	1.3E-04	7.0E-02	7.0E-03
Tramadol	5970	3	0.0015	1.7E-04	1.1E-01	1.5E-04	1.1E+00	1.1E-01
HI					0.84		17.7	1.8

Study three

For study-three, measured concentrations of pharmaceuticals from treated drinking water in several locations in the US were used in the assessment. These data better approximate exposures of humans but

are still worst-case in that the greatest reported concentration for each pharmaceutical was used. The margins of exposure (Table 4) as published by Bruce et al. (2010) were used to calculate HQs and these were then summed as above to give a HI.

Table 4. Hazard quotients for pharmaceuticals in drinking water calculated from data of (Bruce et al. 2010).

Compounds	Exposure (g/kg/d)	Drinking Water Exposure Level	Maximum concentration detected (µg/L)	MOE	HQ
Phenytoin	0.058	2	0.019	110	9.09E-03
Risperidone	0.014	0.49	0.0029	170	5.88E-03
Carbamazepine	0.34	12	0.018	660	1.52E-03
Atenolol	2	70	0.018	3,800	2.63E-04
Meprobamate	7.5	260	0.042	6,200	1.61E-04
Gemfibrozil	0.39	14	0.0021	6,500	1.54E-04
Fluoxetine	0.97	34	0.00082	41,000	2.44E-05
Norfluoxetine	0.97	34	0.00077	44,000	2.27E-05
Diazepam	1	35	0.00033	100,000	1.00E-05
Sulfamethoxazole	510	18,000	0.003	6,000,000	1.67E-07
Atorvastatin	0.14	4.9	<0.00025	>19,000	5.26E-05
Simvastatin	0.14	4.9	<0.00025	>19,000	5.26E-05
Simvastatin hydroxy acid	0.14	4.9	<0.00025	>19,000	5.26E-05
Trimethoprim	190	6,700	<0.00025	>26,000,000	3.85E-08
Enalapril	0.23	8.1	<0.00025	>32,000	3.13E-05
Naproxen	570	20,000	<0.00050	>39,000,000	2.56E-08
Diclofenac	67	2,300	<0.00025	>9,300,000	1.08E-07
o-hydroxy atorvastatin	0.14	4.9	<0.00050	>9,800	1.02E-04
p-hydroxy atorvastatin	0.14	4.9	<0.00050	>9,800	1.02E-04
HI					0.0175

The HI (0.0175) for measured concentrations of pharmaceuticals in drinking water (Table 4) was less than 1. Treatment of drinking water probably resulted in reductions in exposures that further reduced risks from exposures to mixtures. As the likelihood of co-occurrence of high concentrations of pharmaceuticals in a particular time and location is small, the actual risk from the mixtures is smaller than indicated by the HI value.

Study four

Comparison of screening outcomes for a small group of estrogen agonists demonstrates that the approach suggested in the Australian guidelines probably provides the most conservative, but realistic estimate of possible hazard for a mixture of pharmaceuticals in this class. Estimated exposures to estrone, 17-β estradiol, estriol, and 17-α ethinyl estradiol (Caldwell et al. 2010) were used to calculate

Key Points

- Assuming worst-case additivity across classes and co-occurrence of the highest reported concentrations, risks from mixtures of pharmaceuticals measured in treated drinking water were below the level of concern.

HI values using TTC limits, Australian guideline surrogate ADIs (EPHC 2008), and ADIs determined by product manufacturers for their workplaces (Table 5). The highest HI total resulted from using the Australian sADI guideline values. When the potency of the prescribed estrogen agonists was normalized to E2 equivalents, Caldwell et al. (2010) found that the margin of exposure for the mixture was 130 (equivalent to a hazard index total of 0.0077). TTC values appear to significantly underestimate possible hazards.

Table 5. Comparison of hazard index evaluation using TTC, Australian surrogate ADIs, or ADIs from Caldwell et al. (2010)

Estrogen agonists	Drinking water Phate PEC prescription (90% low flow) (Caldwell et al. 2010) in ng/L	Dose from 2 L for 70 kg adult ($\mu\text{g}/\text{kg}/\text{day}$)	Cramer class	TTC w/ Kroes ($\mu\text{g}/\text{kg}/\text{day}$)	Australian guideline sADIs ($\mu\text{g}/\text{kg}/\text{day}$)	Workplace ADIs from Caldwell et al. 2010 ($\mu\text{g}/\text{kg}/\text{day}$)	HQ for TTC	HQ for Australian guideline sADIs	HQ for workplace ADIs
Estrone E1	0.18	5.14E-06	II	9	0.00086	0.001	5.71E-07	0.00598	0.00514
17- β estradiol E2	0.02	5.71E-07	II	9	0.05	0.00029	6.34E-08	1.14E-05	0.00197
Estriol E3	0.000013	3.71E-10	II	9	0.0014	0.001	4.12E-11	2.65E-07	3.71E-07
17- α ethinyl estradiol EE2	0.05	1.42E-06	II	9	0.000043	0.0001	1.58E-07	0.0332	0.0142
HI							7.94E-07	0.0392	0.0214*

*Actual published margin of exposure was based on E2 equivalent exposure to mixture and was 130 (HI of 0.0077).

Study five

The data on concentrations of antibiotics were abstracted from Table 3 and HQs calculated on the basis of the minimum therapeutic dose with an UF of 1000. The HQs were summed to provide a HI (Table 6).

Table 6. Hazard quotients for adults for consumption of antibiotics based on maximum detected concentrations (data from Table 3)

Product	Maximum concentration in surface water (ng/L)	Estimated oral dose (mg/kg/d) based on 2 L/d	Adult therapeutic dose UF=1000	HQ (dose from drinking water/TD with UF=1000)
Amoxicillin	11	3.14E-07	1.07E-02	2.94E-05
Cephalexin	26.8	7.66E-07	1.43E-02	5.35E-05
Roxithromycin	180	5.14E-06	4.29E-03	1.20E-03
Sulfamethoxazole	1340	6.97E-06	1.60E-01	4.36E-05
Ciprofloxacin	0.182	5.20E-09	7.14E-03	7.28E-07
Chlortetracycline	690	4.66E-06	1.43E-02	3.26E-04
Oxytetracycline	47000	1.35E-05	3.33E-03	4.05E-03

Product	Maximum concentration in surface water (ng/L)	Estimated oral dose (mg/kg/d) based on 2 L/d	Adult therapeutic dose UF=1000	HQ (dose from drinking water/TD with UF=1000)
HI				5.70E-03

CONCLUSIONS AND DISCUSSION OF UNCERTAINTIES

There are a number of general conclusions that were reached in this case example:

- Use of a TTC does not appear to always provide the most conservative screening approach for pharmaceuticals.
- Use of the approach published in the Australian guidelines appears to provide a conservative screen for determining surrogate ADIs for pharmaceuticals and is based on readily-available data on therapeutic doses. Given that there is generally more data on the biological activity of pharmaceuticals, the TTC approach may be inappropriate anyway as higher tiers can be just as readily applied.
- Adding HIs within therapeutic classes provides a conservative evaluation of pharmaceutical mixtures.

Assumptions and use of worst-case data for hazards

One of the assumptions in the TTC approach is that the substances assessed fall in a normal distribution with respect to toxicity. This may be appropriate for a large number of general use chemicals but it is questionable if this will be protective where the chemicals (pharmaceuticals) are designed to have biological effects, may be more potent than would be predicted from their basic chemistry, and reside in the lower tail of the effects distribution. Large uncertainty factors are sometimes used to extrapolate from therapeutic doses to ADIs. For example, in the Australian guidelines (EPHC 2008) the lowest therapeutic dose is divided by UF of 1000, with another UF of 10 used for cytotoxic compounds and synthetic or natural hormones. Given that the UF used on the chronic NOEL for the Cramer classes is 100, it is not surprising that the TTC approach is less conservative for certain compounds than the use of UF of 1000 to 10,000.

The TTC approach uses NOELs from chronic studies as a point of departure. The NOEL is based on the observation of *adverse effects* at greater doses. The surrogate ADIs developed for pharmaceuticals are based on a therapeutic dose (e.g., EPHC 2008) and, given that this is, many times, the minimal dose with a physiological effect, the use of large uncertainty factors may often be too conservative.

Assumptions and use of worst-case data for exposures

The assumption of co-occurrence of the highest concentrations in time and space that was used in these assessments represents a worst-case scenario with respect to exposure. The use of this assumption implies that concentrations of all pharmaceuticals in surface waters are highly correlated. While this provides conservatism in terms of the exposures used to calculate the HQ and HI, it is not realistic as it does not consider the probability of co-occurrence. Since all except the first case (above) made use of measured concentrations, access to the raw data would allow HIs to be calculated for a particular day and location. These could be probabilistically analyzed to provide more realistic estimates of the upper centiles of HIs and place risk in perspective.

Assumptions regarding the potential for interactions

It is well known that certain drugs may interact when given at therapeutic doses. These interactions can be due to kinetic and/or dynamic effects that are biochemically and physiologically mediated at therapeutic doses. Given the very small concentrations of individual pharmaceuticals that have been reported in surface waters, the possibility of exposure to effective doses that could result in interactions is judged to be exceedingly small. However, it is hypothetically possible that additivity of concentrations in a class of

chemicals with the same mechanism of action might result in effects on enzymes or transporters that change the kinetics or dynamics of other compounds, resulting in interactive effects.

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