COMBINATION EFFECTS OF CHEMICALS

Some initial perspectives from an environmental NGO

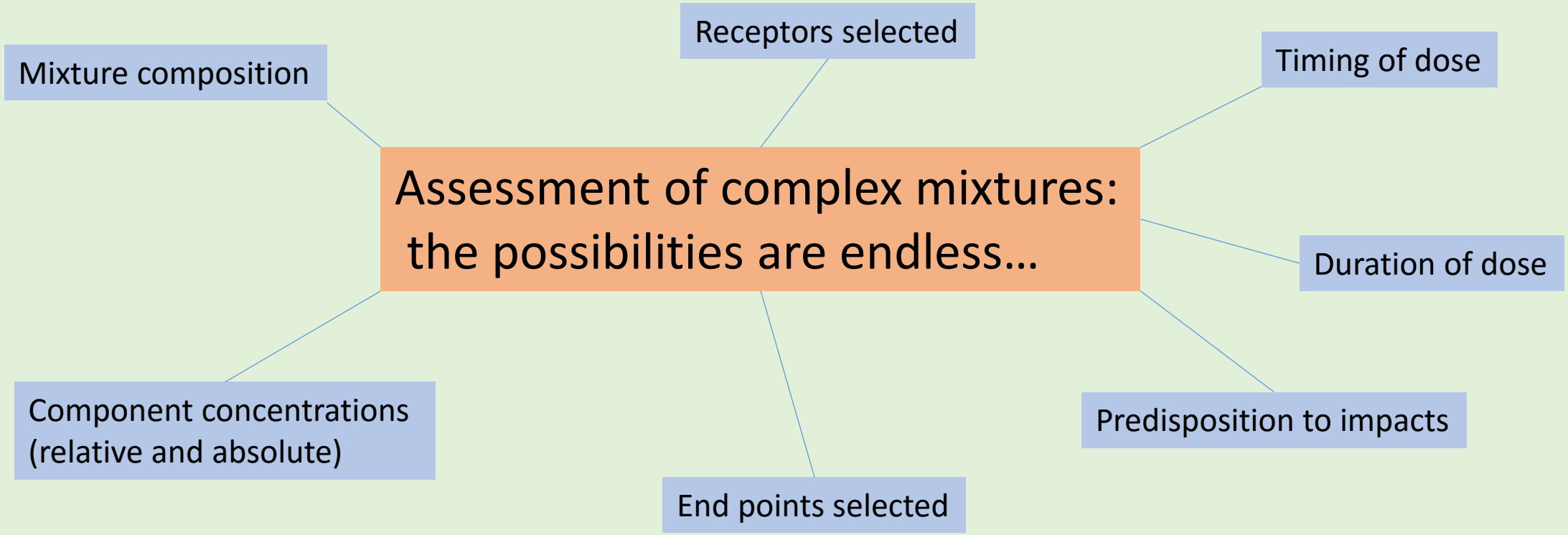
Dr David Santillo

Greenpeace Research Laboratories

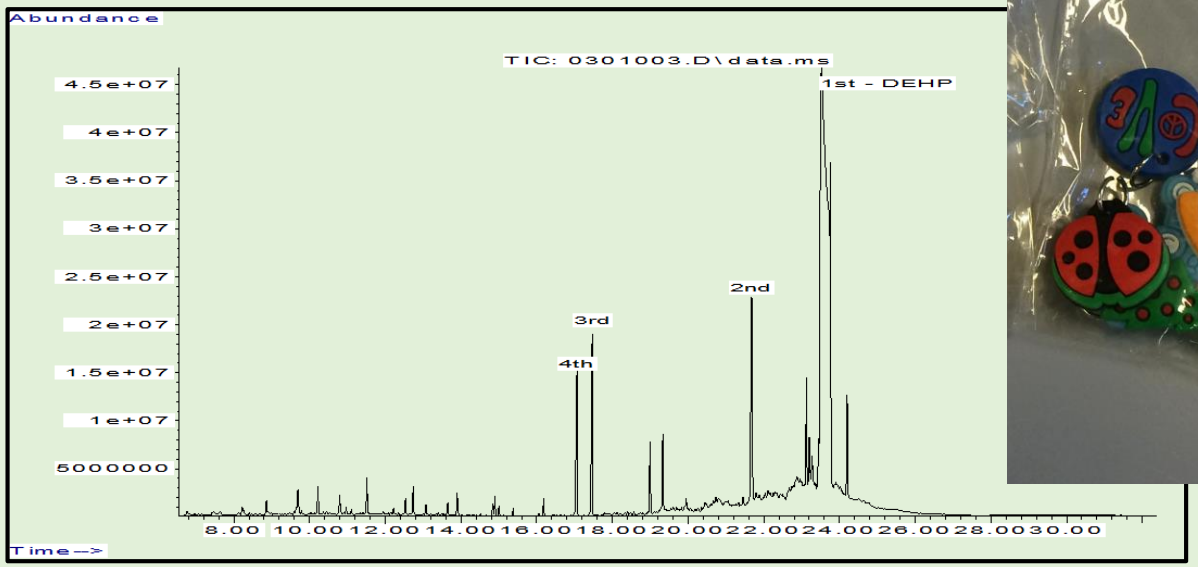
Chemicals in mixtures - general observations

- Chemicals (additives or contaminants) in products, wastes, environmental compartments and foods occur, more often than not, as complex mixtures
- Exposure to complex mixtures should therefore be considered more a norm than an exception
- Toxicity of a mixture may be dominated by one chemical component, but equally may not
- Even assuming additivity models, assessments themselves can quickly become highly complex and uncertain

Chemicals in mixtures - general observations



Chemical complexity in products Loom band charms: Hungary



Number of compounds isolated: 73

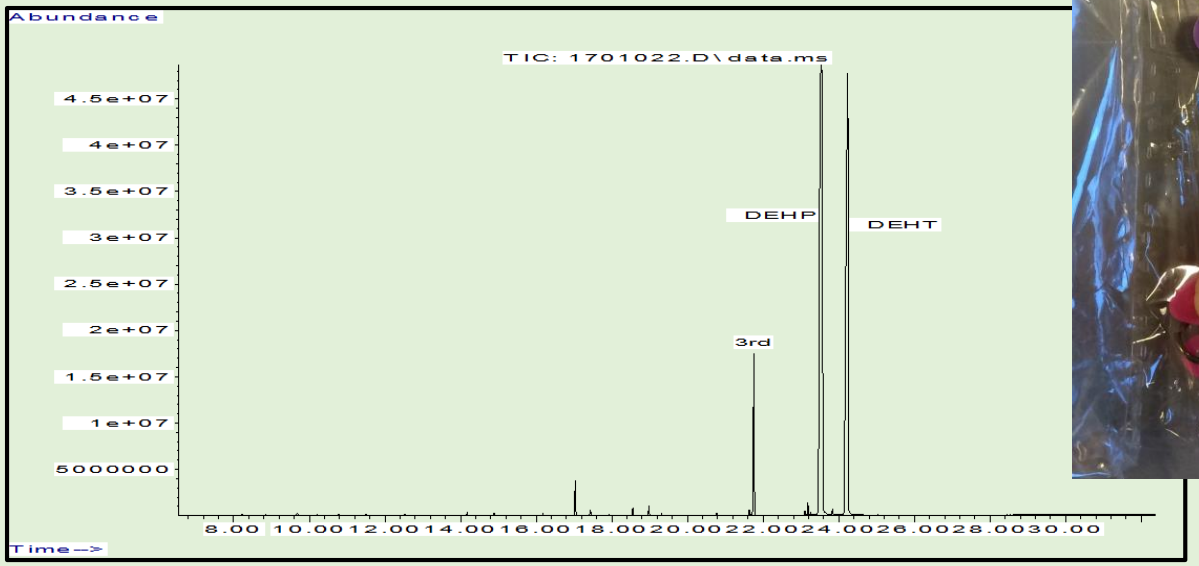
Compounds identified to better than 90%:

CAS#	Name
000117-81-7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (DEHP) 1st
006422-86-2	1,4-Benzenedicarboxylic acid, 1,4-bis(2-ethylhexyl) ester (DEHT)
000872-05-9	1-Decene
036653-82-4	1-Hexadecanol 4th
000112-88-9	1-Octadecene
000629-78-7	Heptadecane
001731-92-6	Heptadecanoic acid, methyl ester
000544-76-3	Hexadecane
000638-36-8	Hexadecane, 2,6,10,14-tetramethyl-
000112-39-0	Hexadecanoic acid, methyl ester 3rd
001750-51-2	Naphthalene, decahydro-1,6-dimethyl-
000629-92-5	Nonadecane
000593-45-3	Octadecane
000112-61-8	Octadecanoic acid, methyl ester
002566-91-8	Oxiraneoctanoic acid, 3-octyl-, methyl ester 2nd
000629-62-9	Pentadecane
001921-70-6	Pentadecane, 2,6,10,14-tetramethyl-
003892-00-0	Pentadecane, 2,6,10-trimethyl-
002882-96-4	Pentadecane, 3-methyl-
000108-95-2	Phenol
000629-59-4	Tetradecane
025117-24-2	Tetradecane, 4-methyl-
000124-10-7	Tetradecanoic acid, methyl ester
000629-50-5	Tridecane

Compounds tentatively identified:

CAS#	Name
071221-49-3	3-Methyl-1-(phenylthio)butan-2-one
002114-42-3	Cyclohexane, 2-propenyl-
017312-57-1	Dodecane, 3-methyl-
000544-85-4	Dotriacontane
054833-23-7	Eicosane, 10-methyl-
001072-05-5	Heptane, 2,6-dimethyl-
017302-32-8	Nonane, 3,7-dimethyl-
000630-07-9	Pentatriacontane
006418-41-3	Tridecane, 3-methyl-
026730-12-1	Tridecane, 4-methyl-
017301-23-4	Undecane, 2,6-dimethyl-

Loom band charms: Philippines



Number of compounds isolated: **54**

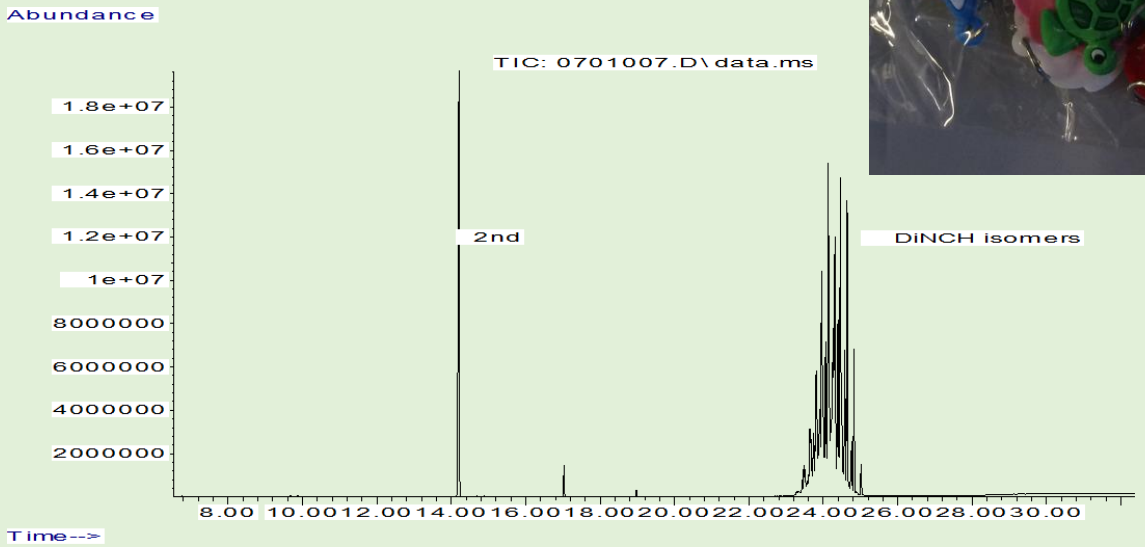
Compounds identified to better than 90%:

CAS#	Name
000117-81-7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (DEHP) 1st
000084-69-5	1,2-Benzenedicarboxylic acid, diisobutyl ester
006422-86-2	1,4-Benzenedicarboxylic acid, 1,4-bis(2-ethylhexyl) ester (DEHT) 2nd
000112-88-9	1-Octadecene
007568-58-3	1-Propene-1,2,3-tricarboxylic acid, tributyl ester
000778-28-9	Benzenesulfonic acid, 4-methyl-, butyl ester
000077-94-1	Butyl citrate
000294-62-2	Cyclododecane
036653-82-4	1-Hexadecanol
000629-78-7	Heptadecane
000544-76-3	Hexadecane
000112-39-0	Hexadecanoic acid, methyl ester
000112-61-8	Methyl stearate
002566-91-8	Oxiraneoctanoic acid, 3-octyl-, methyl ester
000629-62-9	Pentadecane
000630-07-9	Pentatriacontane
000108-95-2	Phenol
000080-05-7	Phenol, 4,4'-(1-methylethylidene)bis-
000629-59-4	Tetradecane
000077-90-7	Tributyl acetylcitrate 3rd
000629-50-5	Tridecane

Compounds tentatively identified:

CAS#	Name
056862-62-5	10-Methylnonadecane
006846-50-0	2-Methyl-propanoic acid, 2,2-dimethyl-1-(1-methylethyl)-1,3-propanediyl
017301-28-9	3,6-Dimethylundecane
005444-75-7	Benzoic acid, 2-ethylhexyl ester
000530-11-8	Benzoic acid, tridecyl ester
017851-53-5	Butyl isobutyl phthalate
001795-15-9	Cyclohexane, octyl-
019879-11-9	Cyclohexanol, 1,2-dimethyl-, cis-
061141-72-8	Dodecane, 4,6-dimethyl-
000631-10-7	Ethyl stearate, 9,12-diepoxy-
000149-57-5	Hexanoic acid, 2-ethyl-
000701-79-1	Oxalic acid, hexyl tetradecyl ester
000833-47-2	Phthalic acid, hex-3-yl pentadecyl ester
025117-24-2	Tetradecane, 4-methyl-
006418-41-3	Tridecane, 3-methyl-

Loom band charms: Germany



Number of compounds isolated: **50**

Compounds identified to better than 90%:

CAS#	Name
006422-86-2	1,2-Cyclohexanedicarboxylic acid, diisononyl ester, 23 isomers 1st
006846-50-0	2-Methyl-propanoic acid, 2,2-dimethyl-1-(1-methylethyl)-1,3-propanediyl ester 2nd
036653-82-4	1-Hexadecanol 3rd
000295-65-8	Cyclohexadecane
000112-34-5	Ethanol, 2-(2-butoxyethoxy)-
084852-15-3	Phenol, nonyl-, 8 isomers
000108-95-2	Phenol

Compounds tentatively identified:

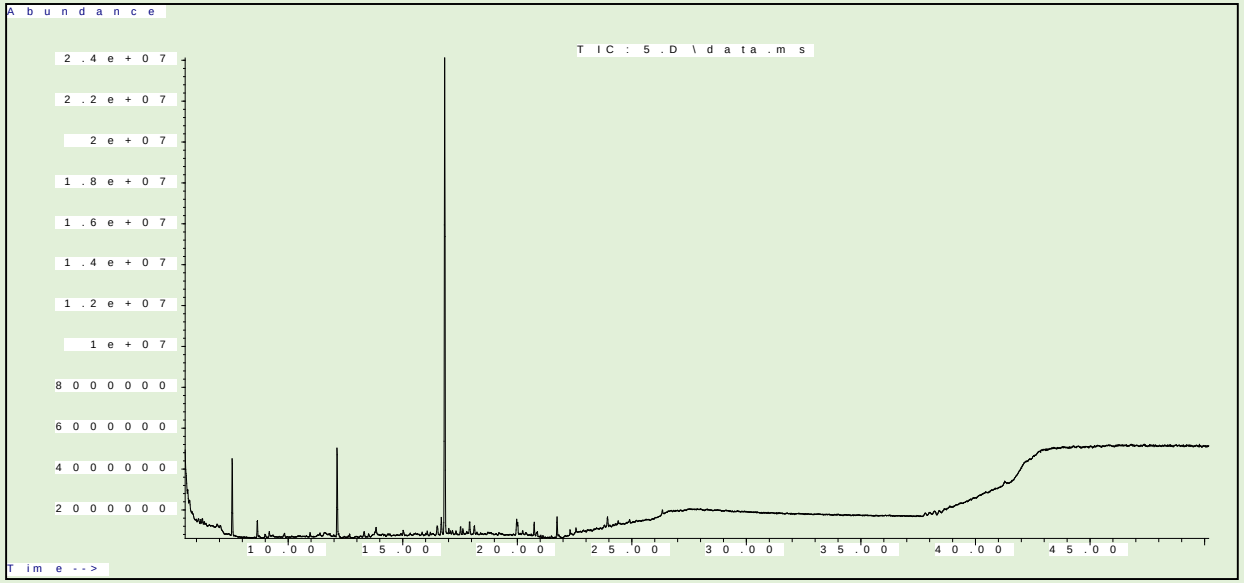
CAS#	Name
000112-70-9	1-Tridecanol

Chemical complexity in wastes Electronics manufacturing facility #1

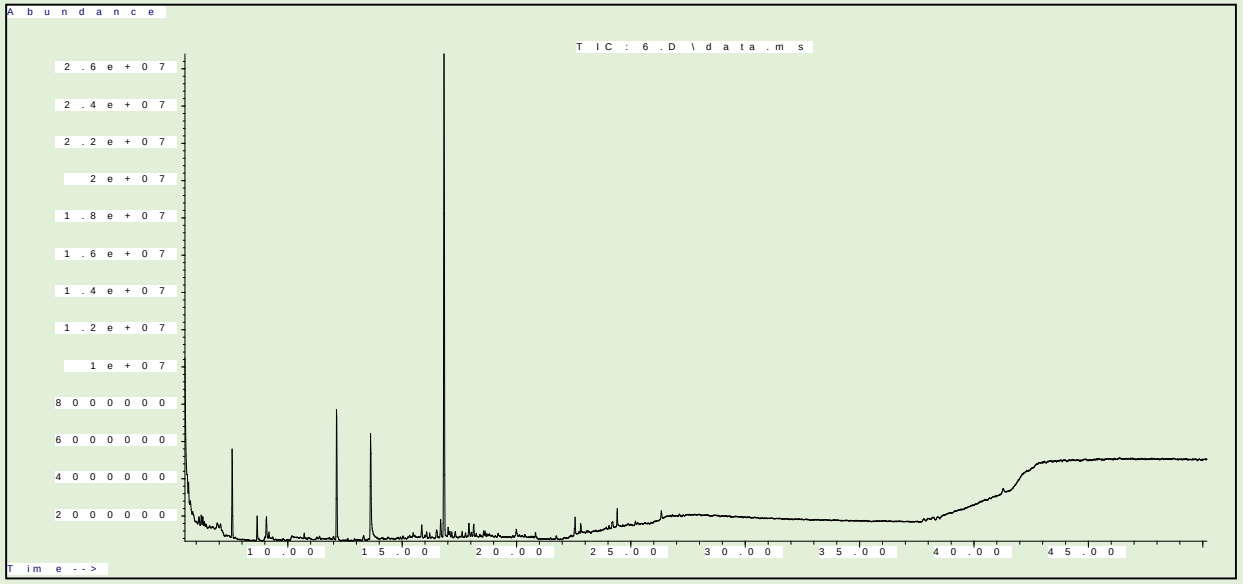
Number of compounds isolated: **31**

Compounds identified to better than 90%: **4**

CAS#	**Name**
000084-66-2	Diethyl phthalate
003622-84-2	Benzenesulfonamide, N-butyl- $$ BM 4
000084-74-2	Di-n-butyl phthalate
000115-86-6	Triphenyl phosphate $$ TPP



Electronics manufacturing facility #2

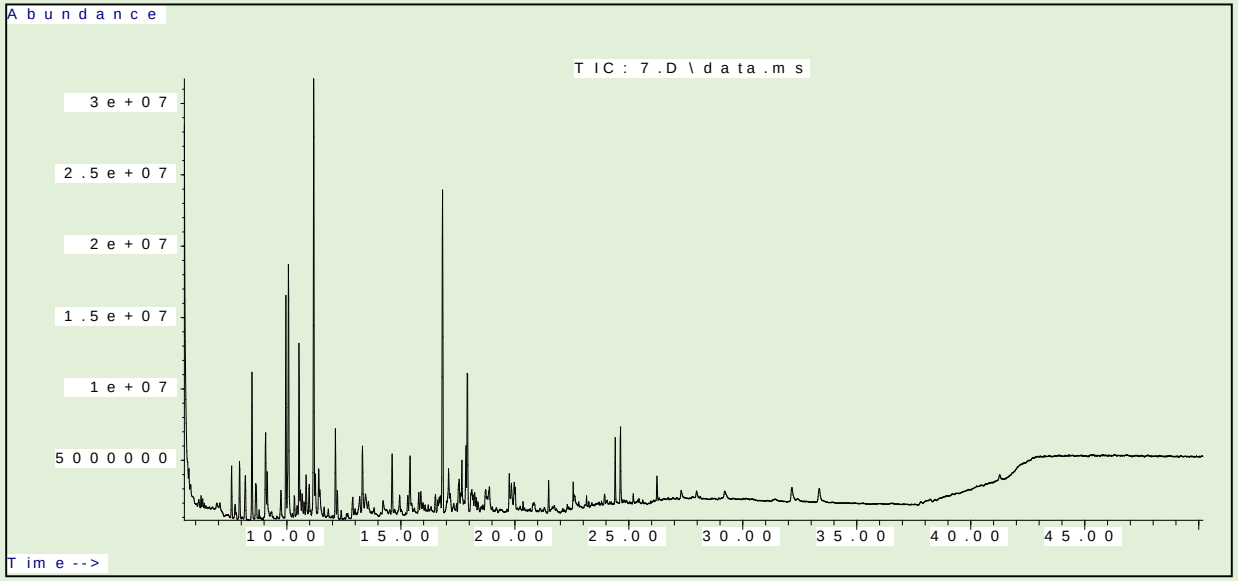


Number of compounds isolated: 30

Compounds identified to better than 90%: 9

CAS#	Name
000065-85-0	Benzoic acid
000126-86-3	2,4,7,9-Tetramethyl-5-decyne-4,7-diol $$ Surfynol 104H
002400-00-2	Benzene, (1-ethyldecyl)-
000084-66-2	1,2-Benzenedicarboxylic acid, diethyl ester $$ DEP
000615-22-5	Benzothiazole, 2-(methylthio)-
013674-84-5	2-Propanol, 1-chloro-, phosphate (3:1) $$ Amgard TMCP
000078-51-3	Ethanol, 2-butoxy-, phosphate (3:1) $$ KP 140
000117-81-7	Bis(2-ethylhexyl)phthalate $$ DEHP
000115-86-6	Triphenyl phosphate $$ TPP

Electronics manufacturing facility #3 (combined sewer)

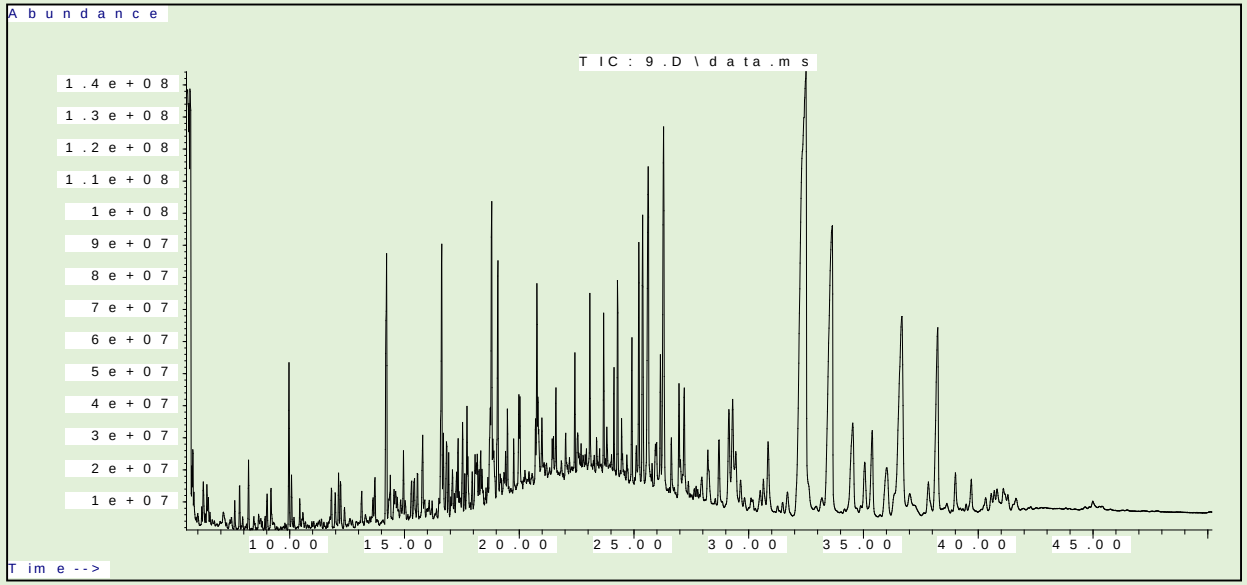


Number of compounds isolated: 146

Compounds identified to better than 90%: 45

CAS#	Name
000000-00-0	p-Menthane, 1,4-epoxy- or p-Menthane, 1,8-epoxy-
000000-00-0	limonene or bornylene
000104-76-7	1-Hexanol, 2-ethyl-
000000-00-0	m-/o-/p-Cymene
018479-58-8	7-Octen-2-ol, 2,6-dimethyl-
000000-00-0	Benzene, dichloro-
000000-00-0	.alpha.-TERPINOLENE or .alpha.-Terpine or carene
000102-82-9	Tributylamine
000078-70-6	1,6-Octadien-3-ol, 3,7-dimethyl-$$ Linalol
000000-00-0	fenchol
000000-00-0	cresol
000000-00-0	cresol, chloro-
000138-87-4	Cyclohexanol, 1-methyl-4-(1-methylethenyl)- $$ p-Menth-8-en-1-ol
000000-00-0	menthol
000000-00-0	Borneol
000000-00-0	Camphor
000000-00-0	Terpineol (2-isomers)
000098-52-2	Cyclohexanol, 4-(1,1-dimethylethyl)-
000000-00-0	Isobornyl acetate or endo-bornyl acetate
000334-48-5	n-Decanoic acid
000089-83-8	Phenol, 5-methyl-2-(1-methylethyl)- $$ Thymol
037275-49-3	4,7-Methano-1H-indenol, hexahydro-
014888-58-5	4,7-Methano-5H-inden-5-one, 3,3a,4,6,7,7a-hexahydro-
000120-72-9	1H-indole $$ 1-aza-indene
000101-84-8	Diphenyl ether
000143-07-7	Dodecanoic acid
000000-00-0	1H-Indole, methyl-
000093-04-9	Naphthalene, 2-methoxy-
000131-11-3	DIMETHYL 0-PHTALATE $$ DMF
000000-00-0	NAPHTHALENE, BROMO-
000000-00-0	4-Methylindoline or Oxindole or o-Tolyl isocyanate
000544-63-8	Tetradecanoic acid
024851-98-7	METHYL (3-OXO-2-PENTYLCYCLOPENTYL) ACETATE
000084-66-2	Diethyl phthalate $$ DEP
000057-10-3	Hexadecanoic acid
000000-00-0	Galaxolide (2 products)
000084-74-2	Dibutyl phthalate $$ DBP
000131-57-7	Oxybenzone $$ Cyasorb uv 9 light absorber
000078-51-3	Tri(2-butoxyethyl) phosphate $$ KP 140
000080-05-7	Phenol, 4,4'-(1-methylethylidene)bis-
000117-81-7	Bis(2-ethylhexyl) phthalate DEHP
000111-02-4	Squalene
000115-86-6	PHENYL PHOSPHATE $$ AI3-04491

Sediment receiving combined sewer wastes



Number of compounds isolated: 253

Compounds identified to better than 90%: 55

CAS#	Name
000124-18-5	Decane
000000-00-0	3-Carene or pinene or .gamma.-Terpinene
001120-21-4	Undecane $$ n-Undecane
000000-00-0	Limonene
000000-00-0	cymene
000099-85-4	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- $$ Crithmene
000000-00-0	Benzene, dichloro-
000586-62-9	Cyclohexene, 1-methyl-4-(1-methylethylidene)-
061141-72-8	Dodecane, 4,6-dimethyl-
000000-00-0	Cresol or Benzenemethanol
000538-68-1	Benzene, pentyl-
000629-50-5	Tridecane
000000-00-0	Tetradecene
001077-16-3	Benzene, hexyl-
000629-59-4	Tetradecane
000629-62-9	Pentadecane
000000-00-0	Longifolene or Isolongifolene
000087-44-5	Caryophyllene
000544-76-3	Hexadecane
000000-00-0	1H-indole or Indolizine
000101-84-8	Benzene, 1,1'-oxybis- $$ DIPHENYL OXIDE $$ GERANIUM CRYSTAL
000128-37-0	Butylated Hydroxytoluene $$ Antioxidant DBPC
000629-78-7	Heptadecane
000000-00-0	1H-Indole, methyl-
004537-15-9	Benzene, (1-butylheptyl)-
004536-86-1	Benzene, (1-propyloctyl)-
004536-87-2	Benzene, (1-ethylnonyl)-
000593-45-3	Octadecane
000000-00-0	2-Pentadecanone or 2-Tridecanone
002719-63-3	Benzene, (1-butyloctyl)-
000629-92-5	Nonadecane
002922-51-2	2-Heptadecanone
000629-94-7	Heneicosane
000000-00-0	galaxolide
000088-29-9	versalide
000084-69-5	DiBP Diisobutyl phthalate
000629-97-0	Docosane
000084-74-2	Dibutyl phthalate $$ DBP
000638-67-5	TRICOSANE
000646-31-1	Tetracosane
000000-00-0	Benzoic acid, heptadecyl ester or Benzoic acid, tetradecyl ester

…etc…

Human exposure through rudimentary electronics recycling

Environment International 74 (2015) 208–220

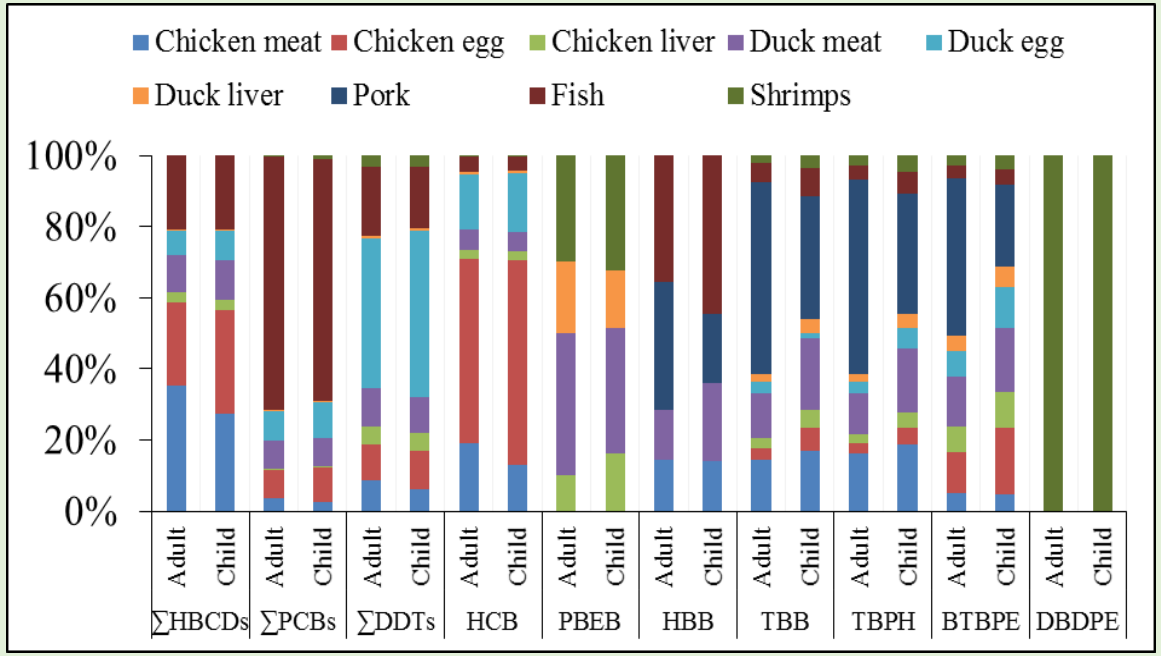
Contents lists available at ScienceDirect

Environment International

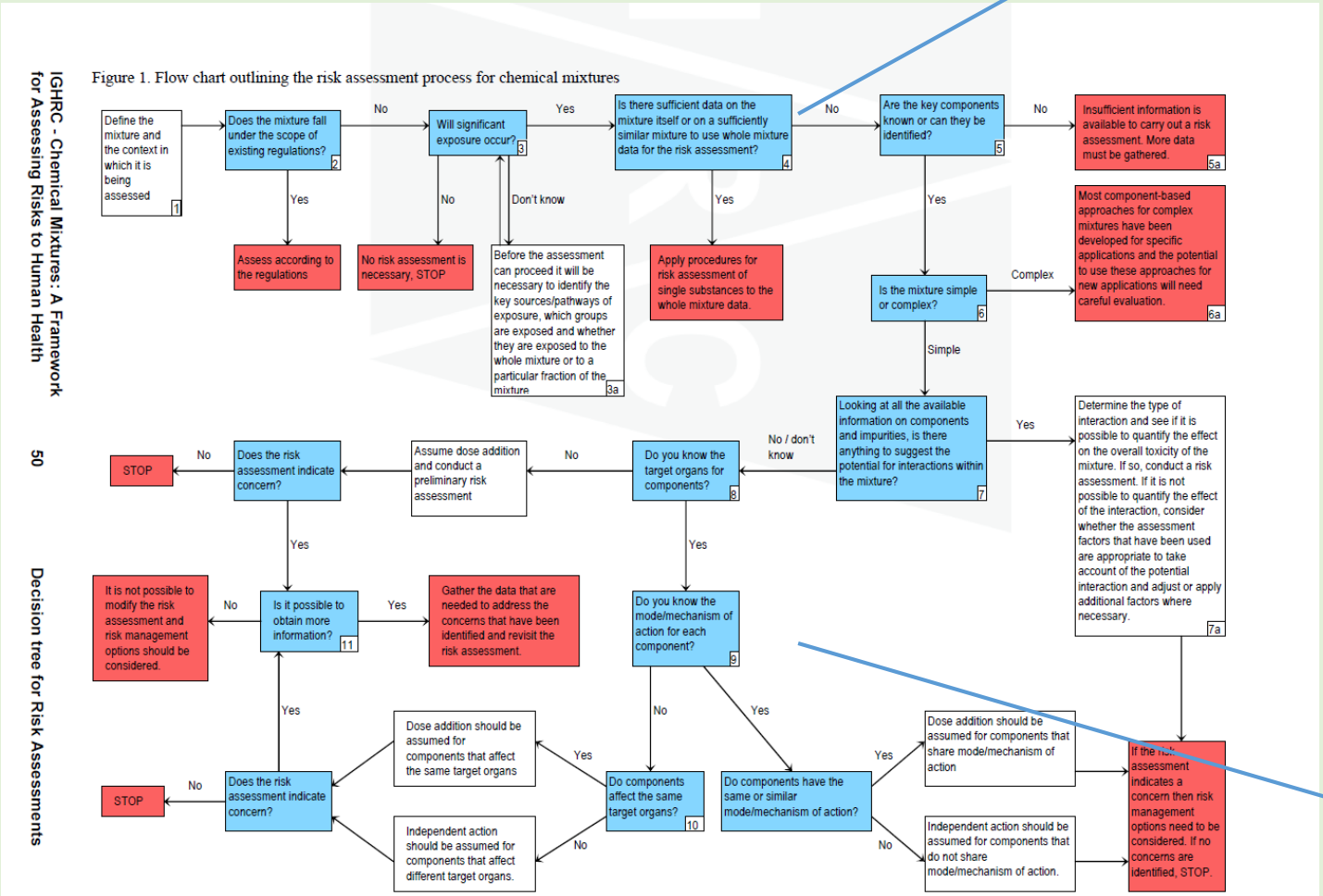
journal homepage: www.elsevier.com/locate/envint

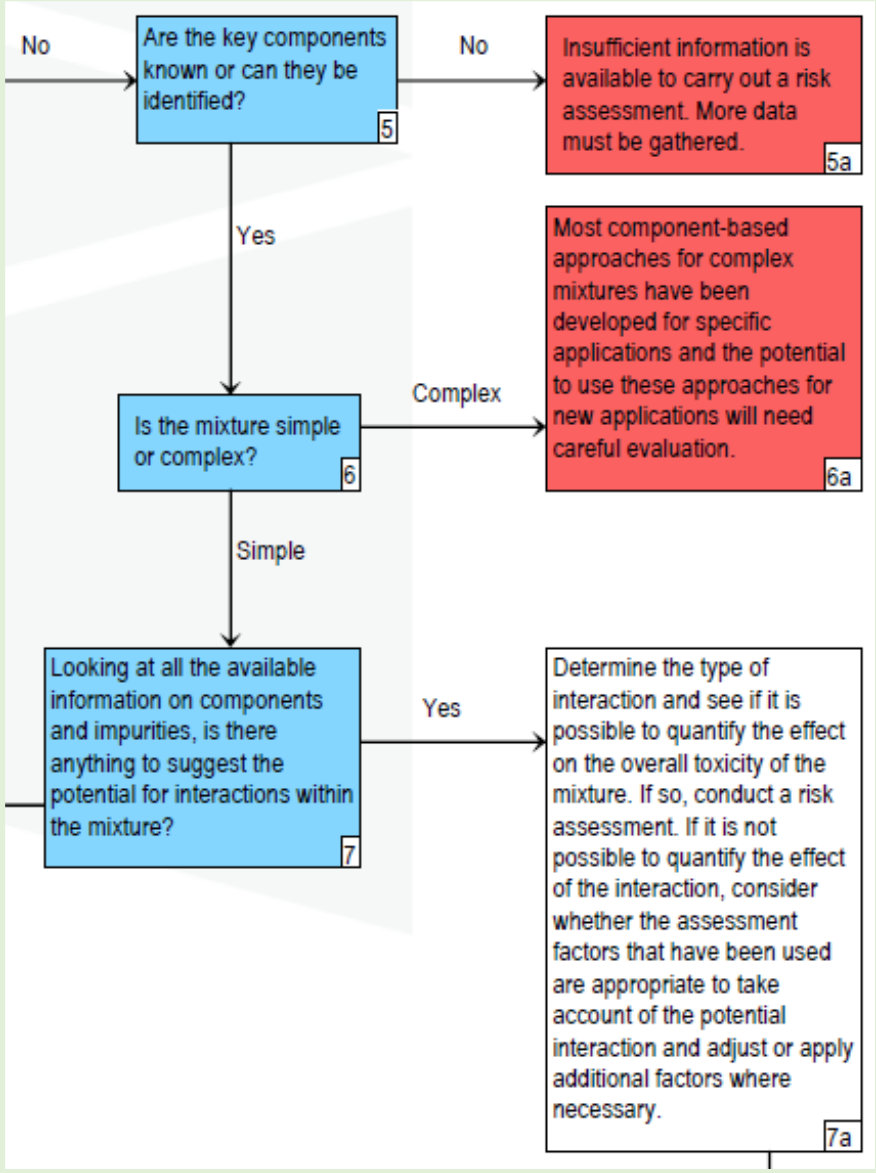
Human dietary intake of organohalogen contaminants at e-waste recycling sites in Eastern China

Iryna Labunska [a,b,*], Mohamed Abou-Elwafa Abdallah [b,c], Igor Eulaers [d,e], Adrian Covaci [d], Fang Tao [b], Mengjiao Wang [a], David Santillo [a], Paul Johnston [a], Stuart Harrad [b]

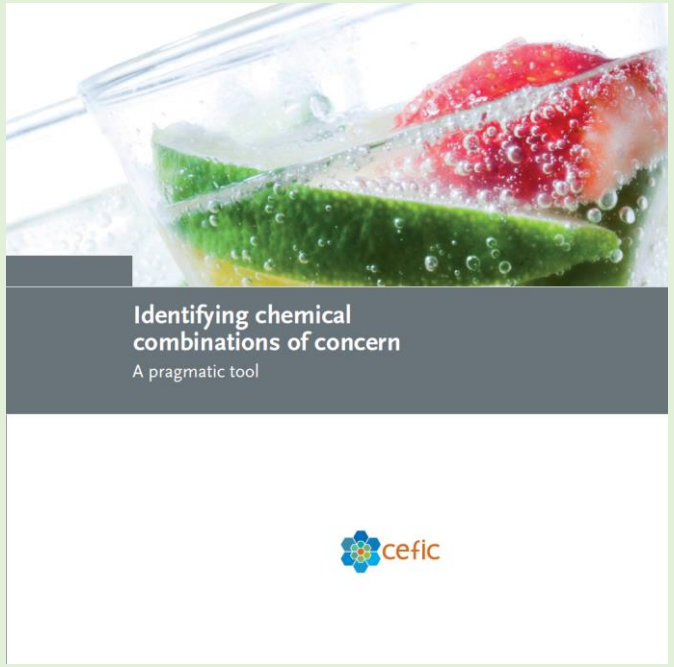


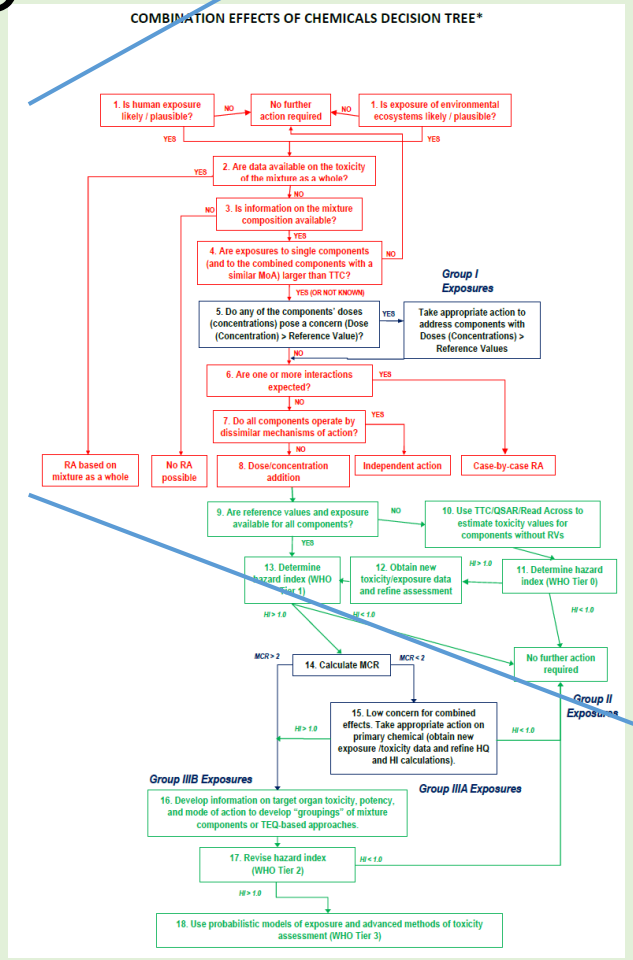
Assessment tools IGHRC 2009

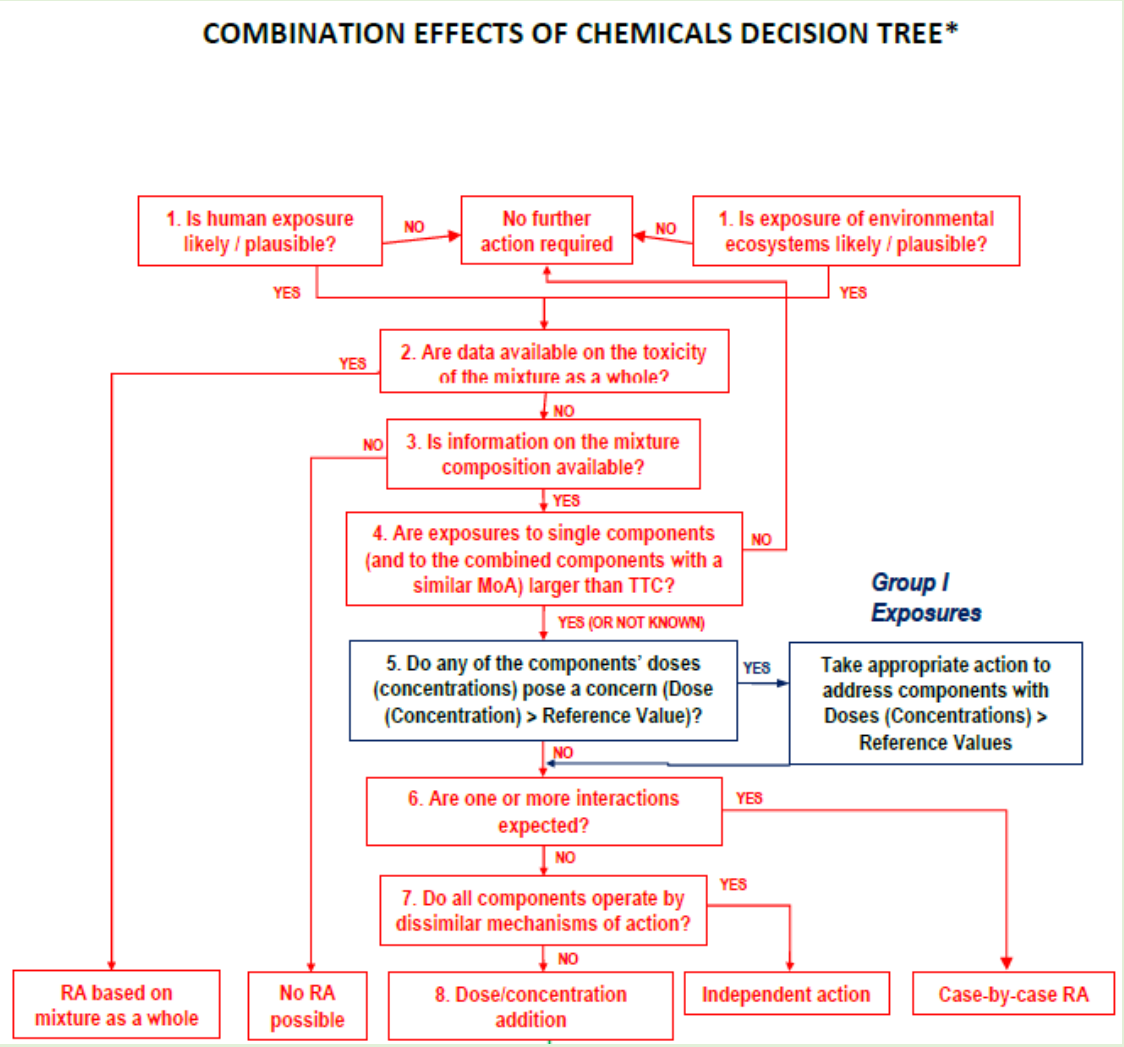




Assessment tools CEFIC 2015







1998

Pergamon

Marine Pollution Bulletin, Vol. 36, No. 12, pp. 939–950, 1998

Printed in Great Britain
0025-326X/98/$19.00+0.00

PII: S0025-326X(98)00113–1

VIEWPOINT

The Precautionary Principle: Protecting Against Failures of Scientific Method and Risk Assessment

D. SANTILLO*‡, R. L. STRINGER*, P. A. JOHNSTON* and J. TICKNER†
*Greenpeace Research Laboratories, Department of Biological Sciences, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, UK
†Work Environment Program, University of Massachusetts Lowell, One University Avenue, Lowell, MA 01854, USA

The precautionary principle has been increasingly incorporated into national and international legislation to protect the environment from anthropogenic impacts. Suggestions for redefinition of the principle have been made (Gray and Bewers, 1996). Adopting these suggestions would entail using a risk-based approach to environmental protection rather than a truly precautionary approach. There remain enormous gaps in our understanding of the identity and of the individual or combined toxicity of chemicals released to the marine environment. This is also true of the actual doses involved in environmental exposures, whereas the most appropriate ways of determining ecosystem impacts have not yet been identified. Classical toxicological assumptions applied to dose–response relationships can no longer be regarded as universally applicable. Ecotoxicology, consequently, contains irresolvable indeterminacies. It is clear, therefore, that the continuing application of the precautionary principle as a paradigm for regulatory action, as a means of recognizing and accounting for limitations to scientific evidence, is an approach that is both scientifically defensible and capable of ensuring a high level of environmental protection. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: precautionary principle; environmental protection; legislation; policies; risks.

The Viewpoint article by Gray and Bewers (1996) represents an interesting, but misguided, contribution to the long-running debate surrounding the precautionary principle. The authors suggest that the application of the concept should be extended to address physical impacts rather than only those that are related to chemical inputs. They argue, also, that the definition of the precautionary principle should explicitly endorse a role for science. To be clear at the outset, application of the precautionary principle to non-chemical impacts does not require a redefinition of the principle itself. Further, there is obviously a role for scientific activity in marine environmental regulation, management and protection. Indeed, we would wholeheartedly concur with Gray and Bewers observation and the enthusiastic subsequent endorsement of this by Chapman (1997) that marine environmental protection activities require more science rather than less. There is a pressing need to increase our understanding of marine systems in order to formulate effective regulation, to implement it and subsequently to assess its effectiveness. This implies increased research effort and suitable channeling of adequate resources. It must be acknowledged, however, that in many cases, the existing scientific data base cannot be used to justify a particular course of action, or indeed to justify taking no action at all. In this context, a decision not to act in the face of uncertainty is as much a policy decision as taking precautionary action. In these situations, precaution is needed. This keystone principle has gained widespread acceptance internationally since the late 1980s.

Regulation and Definitions

It was recognition of the limitations of scientific knowledge that led originally to the formulation of an approach to environmental protection which was fundamentally precautionary in nature. Broadly speaking, such an approach recognizes limitations to scientific and technical information and promotes

‡Corresponding author.

2013

Martin *et al. Environmental Health* 2013, **12**:53
http://www.ehjournal.net/content/12/1/53

ENVIRONMENTAL HEALTH

REVIEW Open Access

Dispelling urban myths about default uncertainty factors in chemical risk assessment – sufficient protection against mixture effects?

Olwenn V Martin, Scholze Martin and Andreas Kortenkamp*

Abstract

Assessing the detrimental health effects of chemicals requires the extrapolation of experimental data in animals to human populations. This is achieved by applying a default uncertainty factor of 100 to doses not found to be associated with observable effects in laboratory animals. It is commonly assumed that the toxicokinetic and toxicodynamic sub-components of this default uncertainty factor represent worst-case scenarios and that the multiplication of those components yields conservative estimates of safe levels for humans. It is sometimes claimed that this conservatism also offers adequate protection from mixture effects. By analysing the evolution of uncertainty factors from a historical perspective, we expose that the default factor and its sub-components are intended to represent adequate rather than worst-case scenarios. The intention of using assessment factors for mixture effects was abandoned thirty years ago. It is also often ignored that the conservatism (or otherwise) of uncertainty factors can only be considered in relation to a defined level of protection. A protection equivalent to an effect magnitude of 0.001-0.0001% over background incidence is generally considered acceptable. However, it is impossible to say whether this level of protection is in fact realised with the tolerable doses that are derived by employing uncertainty factors. Accordingly, it is difficult to assess whether uncertainty factors overestimate or underestimate the sensitivity differences in human populations. It is also often not appreciated that the outcome of probabilistic approaches to the multiplication of sub-factors is dependent on the choice of probability distributions. Therefore, the idea that default uncertainty factors are overly conservative worst-case scenarios which can account both for the lack of statistical power in animal experiments and protect against potential mixture effects is ill-founded. We contend that precautionary regulation should provide an incentive to generate better data and recommend adopting a pragmatic, but scientifically better founded approach to mixture risk assessment.

Keywords: Acceptable daily intake, Extrapolation, Hazard assessment, Mixture, Risk assessment

Background

Predicting the potential detrimental effects on human health of toxicants requires the extrapolation of experimental data in animals to the general population. This is routinely conducted by dividing the highest dose not exhibiting observable toxicity, no observed adverse effect levels (NOAELs), by a default uncertainty factor of 100.

In the years preceding the implementation of the European Community Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (EC 1907/2006) (REACH) in June 2007, a number of extensive reviews of the literature concerning uncertainty factors were published by government agencies and industry associations [1-5]. Recent developments regarding our understanding of the differences in sensitivity to the toxic effects of chemicals between species and between individuals alone render a re-analysis both timely and necessary. This is also now crucial because a number of myths about the supposed properties of uncertainty factors do persist and these might have serious implications for the regulation of mixtures. Principally, it is often assumed that default uncertainty factors represent worst-case scenarios and that the multiplication of their component factors yields sufficiently conservative, or as some would argue, even overly conservative

* Correspondence: andreas.kortenkamp@brunel.a.cuk
Institute for the Environment, Brunel University, Kingston Lane, Uxbridge UB8 3PH, United Kingdom

Can uncertainty / safety factors fill the gaps for combination effects of mixtures?

"Pessimistic assumptions […] are, therefore, likely to be no more reliable than optimistic assumptions or, indeed, capable of being differentiated from them."

"[…] default uncertainty factors do not represent worst-case scenarios and were not intended to do so […] the data available does not support an unwavering belief in the purported conservativeness of the overall default uncertainty factor of 100."

Testing interactions in binary mixtures: metals and PFAAs

Ecotoxicology and Environmental Safety 122 (2015) 37–44

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Statistically significant deviations from additivity: What do they mean in assessing toxicity of mixtures?

Yang Liu [a,*], Martina G. Vijver [a], Hao Qiu [a], Jan Baas [b], Willie J.G.M. Peijnenburg [a,c]

[a] Institute of Environmental Sciences (CML), Leiden University, 2300 RA Leiden, The Netherlands
[b] Centre for Ecology & Hydrology (CEH), MacLean Building, Benson Lane, OX10 8BB Wallingford, Oxfordshire, United Kingdom
[c] National Institute of Public Health and the Environment (RIVM), Center for Safety of Substances and Products, 3720 BA Bilthoven, The Netherlands

ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form
7 July 2015
Accepted 8 July 2015
Available online 17 July 2015

Keywords:
Metal mixtures
Lettuce
Statistically significant
Biologically relevant
Reproducibility

ABSTRACT

There is increasing attention from scientists and policy makers to the joint effects of multiple metals on organisms when present in a mixture. Using root elongation of lettuce (*Lactuca sativa* L.) as a toxicity endpoint, the combined effects of binary mixtures of Cu, Cd, and Ni were studied. The statistical MixTox model was used to search deviations from the reference models i.e. concentration addition (CA) and independent action (IA). The deviations were subsequently interpreted as 'interactions'. A comprehensive experiment was designed to test the reproducibility of the 'interactions'. The results showed that the toxicity of binary metal mixtures was equally well predicted by both reference models. We found statistically significant 'interactions' in four of the five total datasets. However, the patterns of 'interactions' were found to be inconsistent or even contradictory across the different independent experiments. It is recommended that a statistically significant 'interaction', must be treated with care and is not necessarily biologically relevant. Searching a statistically significant interaction can be the starting point for further measurements and modeling to advance the understanding of underlying mechanisms and non-additive interactions occurring inside the organisms.

1. Introduction

Industrial discharges, consumer wastes and the usage of plant protection products or sewage sludge bio-fertilizers may all lead to metal contamination in soil. Metals can be easily adsorbed in soils (Yang et al., 2009) and be accumulated in plants which may result in a threat to the health of the plant itself and consumers in the food chain. In the natural environment, plants are often exposed to multiple metals simultaneously rather than a single metal (Backhaus et al., 2000). Many metals listed individually within the safe range of industrial permits are extremely toxic to certain species and even more so when present in combination (Wong et al., 1987). Thus, to maintain healthy and functioning ecosystems, it is necessary to improve the understanding of combined effects of multiple metals on terrestrial plants.

Since testing is cost- and time-consuming, computational models are developed to help predict toxicological responses and understand the toxicity mechanisms of mixtures. The most frequently used predictive tools for assessing mixture toxicity disregarding interactions are concentration addition (CA) (Loewe and Muischnek, 1926) and independent action (IA) (Bliss, 1939) if the constituents making up the mixture are known. The CA model is used for chemical mixtures for which a similar mode of action is assumed, whereas the IA model is used to predict effects of compounds with a different mode of action (Bliss, 1939). It has been argued that concentration addition should be a more suited default model in risk assessment of chemical mixtures because of its conservatism in most cases (Cedergreen et al., 2008). In addition, it is suggested that dissimilarly acting chemicals rarely exist in complex organisms (Faust et al., 2003). However, the sites or the modes of action are ambiguously defined at the biochemical level and can be dose dependent (Cedergreen et al., 2008). In most cases, the CA and the IA models are used only based on their mathematical connotation as the toxicity mechanisms of metals are still greatly unknown. The conceptually unrelated CA and IA models are single-time point approaches which make them suitable to make predictions for mixture effects based on standardized toxicological tests. Therefore, the CA and the IA models were both used in this research. An elaborate description of these two approaches can be found for example in the papers by Altenburger et al. (2000) and Jonker et al. (2005).

Predicting mixture effects becomes a challenge when a mixture is composed of interacting chemicals that synergize or antagonize the effects of each other. Accurately determining chemical

* Corresponding author.
E-mail address: liu@cml.leidenuniv.nl (Y. Liu).

http://dx.doi.org/10.1016/j.ecoenv.2015.07.012
0147-6513/

Toxicology 316 (2014) 43–54

Contents lists available at ScienceDirect

Toxicology

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

Evaluating the additivity of perfluoroalkyl acids in binary combinations on peroxisome proliferator-activated receptor-α activation☆

Cynthia J. Wolf [a,*], Cynthia V. Rider [b], Christopher Lau [a], Barbara D. Abbott [a]

[a] Developmental Toxicology Branch, Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, ORD, U.S. EPA, Research Triangle Park, NC 27711, United States
[b] National Toxicology Program, NIEHS, Research Triangle Park, NC 27709, United States

ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form
18 November 2013
Accepted 7 December 2013
Available online 26 December 2013

Keywords:
Perfluoroalkyl acids
PFOA
Mixtures
Additivity
PPARα

ABSTRACT

Perfluoroalkyl acids (PFAAs) are found globally in the environment, detected in humans and wildlife, and are typically present as mixtures of PFAA congeners. Mechanistic studies have found that responses to PFAAs are mediated in part by PPARα. Our previous studies showed that individual PFAAs activate PPARα transfected into COS-1 cells. The goal of the current study was to determine if binary combinations of perfluorooctanoic acid (PFOA) and another PFAA act in an additive fashion to activate PPARα in the mouse one-hybrid *in vitro* model. COS-1 cells were transiently transfected with mouse PPARα luciferase reporter construct and exposed to either vehicle control (0.1% DMSO or water), PPARα agonist (WY14643, 10 μM), PFOA at 1–128 μM, perfluorononanoic acid (PFNA) at 1–128 μM, perfluorohexanoic acid (PFHxA) at 8–1024 μM, perfluorooctane sulfonate (PFOS) at 4–384 μM or perfluorohexane sulfonate (PFHxS) at 8–2048 μM to generate sigmoidal concentration–response curves. In addition, cells were exposed to binary combinations of PFOA + either PFNA, PFHxA, PFOS or PFHxS in an 8 × 8 factorial design. The concentration–response data for individual chemicals were fit to sigmoidal curves and analyzed with nonlinear regression to generate EC_{50}s and Hillslopes, which were used in response-addition and concentration–addition models to calculate predicted responses for mixtures in the same plate. All PFOA + PFAA combinations produced concentration–response curves that were closely aligned with the predicted curves for both response addition and concentration addition at low concentrations. However, at higher concentrations of all chemicals, the observed response curves deviated from the predicted models of additivity. We conclude that binary combinations of PFAAs behave additively at the lower concentration ranges in activating PPARα in this *in vitro* system.

Published by Elsevier Ireland Ltd.

1. Introduction

Humans are exposed to a multitude of environmental contaminants. Many environmental contaminants are found not as a single chemical, but as a group of chemicals of the same family. Perfluoroalkyl acids (PFAAs) are one such class of chemicals. Assessing the health risks of PFAAs must take into consideration the joint action of their mixtures.

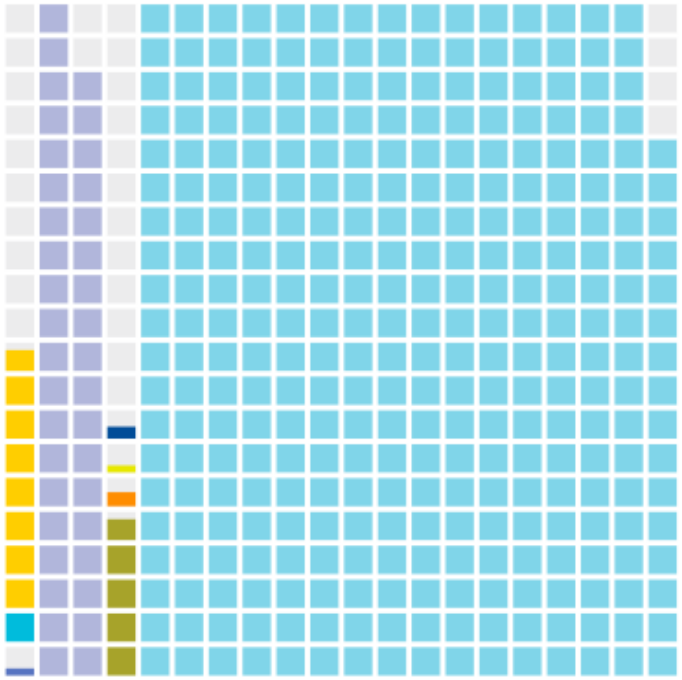
PFAAs are man-made surfactants found globally in the environment and in wildlife and human serum as a mixture of individual members of the PFAA class (Lau et al., 2007). These chemicals are ubiquitous as they are found in many consumer and industrial applications, including oil-resistant coatings on food packaging, water-repellants, fire-fighting foams, industrial lubricants, and cosmetics (Kissa, 2001; Renner, 2001) and are generated by degradation of fluoropolymers. PFAAs are present as mixtures in environmental media such as food, water, air and house dust (Eschauzier et al., 2013; Fromme et al., 2007; Vestergren et al., 2012), and in human serum (Calafat et al., 2007; Ode et al., 2013). Manufacturing has ceased for perfluorooctane sulfonate (PFOS) in the U.S. and has been scaled down for some other PFAAs worldwide (Buck et al., 2011; Lau et al., 2007), and this is reflected in the significant decline in levels of PFOS in human serum in the past decade from 30.4 ng/mL to 13.2 ng/mL (Calafat et al., 2007; Kato et al., 2011). However, these levels are still high compared to other PFAAs. Also, this class of chemicals is very stable and levels of PFOS

☆ *Disclaimer:* This paper has been reviewed by the National Health and Environmental Effects Research Laboratory, US EPA. The use of trade names is for identification only and does not constitute endorsement by the US EPA. The findings and conclusions in this report are those of the authors and do not necessarily reflect the views of the US EPA.

* Corresponding author at: US EPA, NHEERL, TAD, MD-67, 109 TW Alexander Drive, Research Triangle Park, NC 27711, United States. Tel.: +1 919 541 5195; fax: +1 919 541 4017.
E-mail address: wolf.cynthiaj@epa.gov (C.J. Wolf).

0300-483X/$ – see front matter. Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.tox.2013.12.002

Norrona
Lofoten Gore-tex pro jacket (Norway)



Sum PFC **730 µg/m²**

PFC	Concentration in µg/m²	Percentage
PFBS	0.21	0.03
PFBA	1.72	0.2
PFPA	10.6	1.8
PFHxA	76.4	10
PFHpA	9.97	1.20
PFOA	0.67	0.1
PFNA	0.11	0.02
PFDA	0.31	0.04
6:2 FTOH	630	86.40

Patagonia
Men's Super Alpine Jacket (Taiwan)

Sum PFC **284 µg/m²**

PFC	Concentration in µg/m²	Percentage
PFBS	28.9	10
PFBA	18.2	6.5
PFPA	3.79	1.4
PFHxA	25.1	8.9
PFHpA	21.3	7.5
PFOA	0.19	0.1
PFDA	0.07	0.02
6:2 FTOH	180	63
10:2 FTOH	6.7	2.4

Country	Sample type	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	(PFTrA	PFTA	PFBS	PFHxS	PFHpS	PFOS	PFDeS	H4PFOS	8:2 FTS	PFOSA
Russia Altay Mountains	water	**0,605**	< 0,034	< 0,025	**0,174**	**0,173**	**0,151**	**0,039**	**0,037**	< 0,010	< 0,039	< 0,008	< 0,010	< 0,054	< 0,034	**0,010**	< 0,005	< 10,17	< 0,033	< 0,067
	field blank	< 0,495	< 0,065	< 0,049	< 0,026	**0,170**	< 0,082	< 0,026	< 0,035	< 0,019	< 0,075	< 0,015	< 0,020	< 0,104	< 0,064	< 0,015	< 0,010	< 19,36	< 0,064	< 0,128
China Haba Snow Mountains	water	< 0,233	< 0,030	< 0,023	< 0,012	< 0,046	< 0,038	< 0,012	< 0,016	< 0,009	< 0,035	< 0,007	< 0,009	< 0,048	< 0,030	< 0,007	< 0,004	< 9,100	< 0,030	< 0,060
	field blank	< 0,526	**0,732**	**0,062**	**0,179**	**0,338**	< 0,087	**0,030**	< 0,037	< 0,021	< 0,079	< 0,016	< 0,021	< 0,110	< 0,068	0,020	< 0,011	< 20,57	< 0,068	< 0,136
Slovakia High Tatras	water	< 0,521	**0,216**	**0,083**	**0,137**	**0,191**	**0,117**	**0,047**	**0,051**	< 0,020	< 0,078	< 0,016	< 0,021	< 0,109	< 0,068	**0,030**	< 0,011	< 20,35	< 0,067	< 0,134
	water	< 0,526	**0,121**	**0,087**	**0,096**	**0,190**	**0,118**	**0,048**	**0,052**	**0,024**	< 0,079	**0,021**	< 0,021	< 0,110	< 0,068	< 0,016	< 0,011	< 20,57	< 0,068	< 0,136
	field blank	< 0,526	< 0,069	< 0,052	< 0,027	**0,470**	< 0,087	< 0,028	< 0,037	< 0,021	< 0,079	< 0,016	< 0,021	< 0,110	<0,068	< 0,016	< 0,011	< 20,57	< 0,068	< 0, 136
Switzerland Alps/Lake Macun	water	**0,773**	< 0,062	**0,156**	**0,326**	**0,561**	**0,233**	**0,051**	< 0,033	< 0,018	< 0,071	< 0,015	**0,075**	< 0,099	< 0,061	**0,089**	< 0,010	< 18,45	< 0,061	< 0,122
	water	< 0,481	< 0,063	**0,127**	**0,225**	**0,355**	**0,140**	**0,048**	< 0,034	< 0,019	< 0,072	< 0,015	**0,056**	< 0,101	< 0,062	**0,053**	< 0,010	< 18,80	< 0,062	< 0,124
	field blank	< 0,477	< 0,062	< 0,047	< 0,025	**0,248**	< 0,079	< 0,025	< 0,034	< 0,019	< 0,072	< 0,015	< 0,019	< 0,100	< 0,062	< 0,015	< 0,010	< 18,62	< 0,061	< 0,123
Chile Patagonia Torres del Paine	water	**1,118**	< 0,038	**0,038**	**0,025**	< 0,057	< 0,048	< 0,015	< 0,020	< 0,011	< 0,044	< 0,009	< 0,012	< 0,061	< 0,038	< 0,009	< 0,006	< 11,41	< 0,037	< 0,075
	field blank	< 0,538	< 0,070	< 0,053	< 0,028	< 0,106	< 0,089	< 0,028	< 0,038	< 0,021	< 0,081	< 0,017	< 0,022	< 0,112	< 0,070	< 0,017	< 0,011	< 21,00	< 0,069	< 0,139
Italy Appeninnes Lago di Pilato	water	< 0,468	< 0,061	< 0,046	**0,084**	< 0,092	< 0,078	**0,035**	< 0,033	< 0,018	< 0,070	< 0,014	**0,031**	< 0,098	< 0,061	< 0,015	< 0,009	< 18,28	< 0,060	< 0,120
	field blank	< 0,532	< 0,069	< 0,052	< 0,028	< 0,105	< 0,088	< 0,028	< 0,038	< 0,021	< 0,080	< 0,017	< 0,021	< 0,111	< 0,069	< 0,017	< 0,011	< 20,78	< 0,068	< 0,137
Turkey Kaçkar Mountains	water	< 0,495	< 0,065	**0,070**	**0,100**	**0,113**	< 0,082	**0,041**	< 0,035	< 0,019	< 0,075	< 0,015	**0,034**	< 0,104	< 0,064	< 0,015	< 0,010	< 19,36	< 0,064	< 0,128

Tendency towards higher complexity in environmental risk assessment of Plant Protection Products: to accept or to avoid?

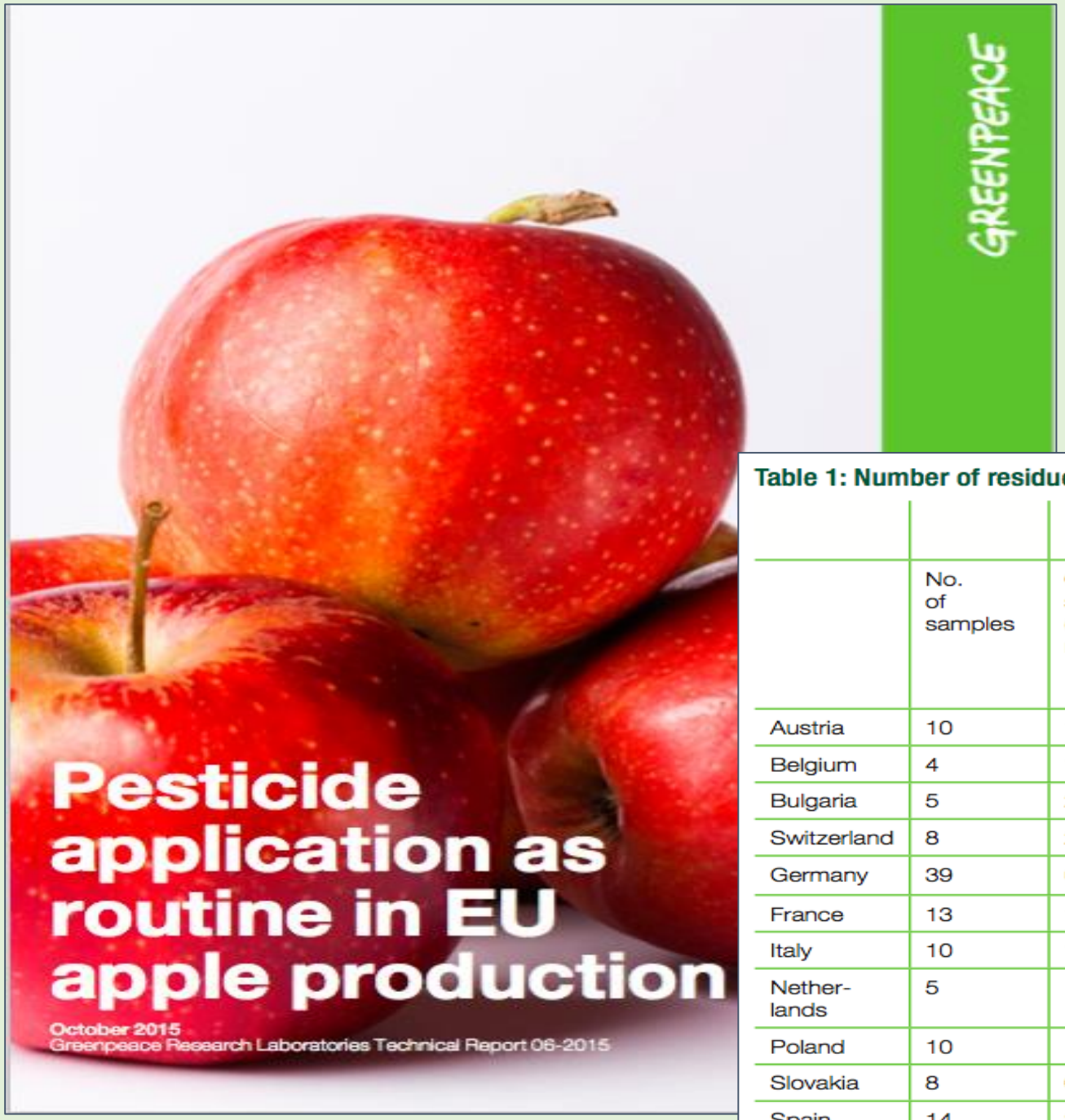
Key messages from an NGO perspective

Mengjiao Wang, David Santillo & Paul Johnston
Greenpeace Research Laboratories
Innovation Centre, University of Exeter, UK

Nantes 2016
SETAC Europe

SETAC Europe 26th Annual Meeting, Nantes, France, 22-26 May 2016

GREENPEACE

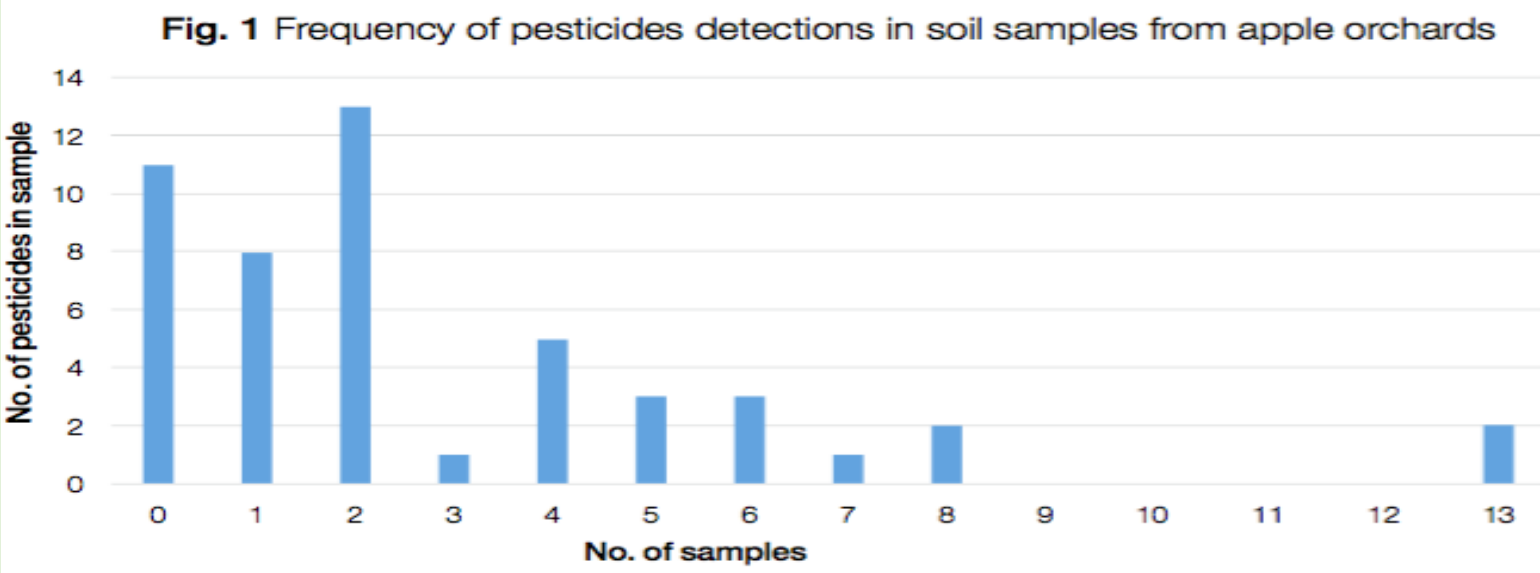


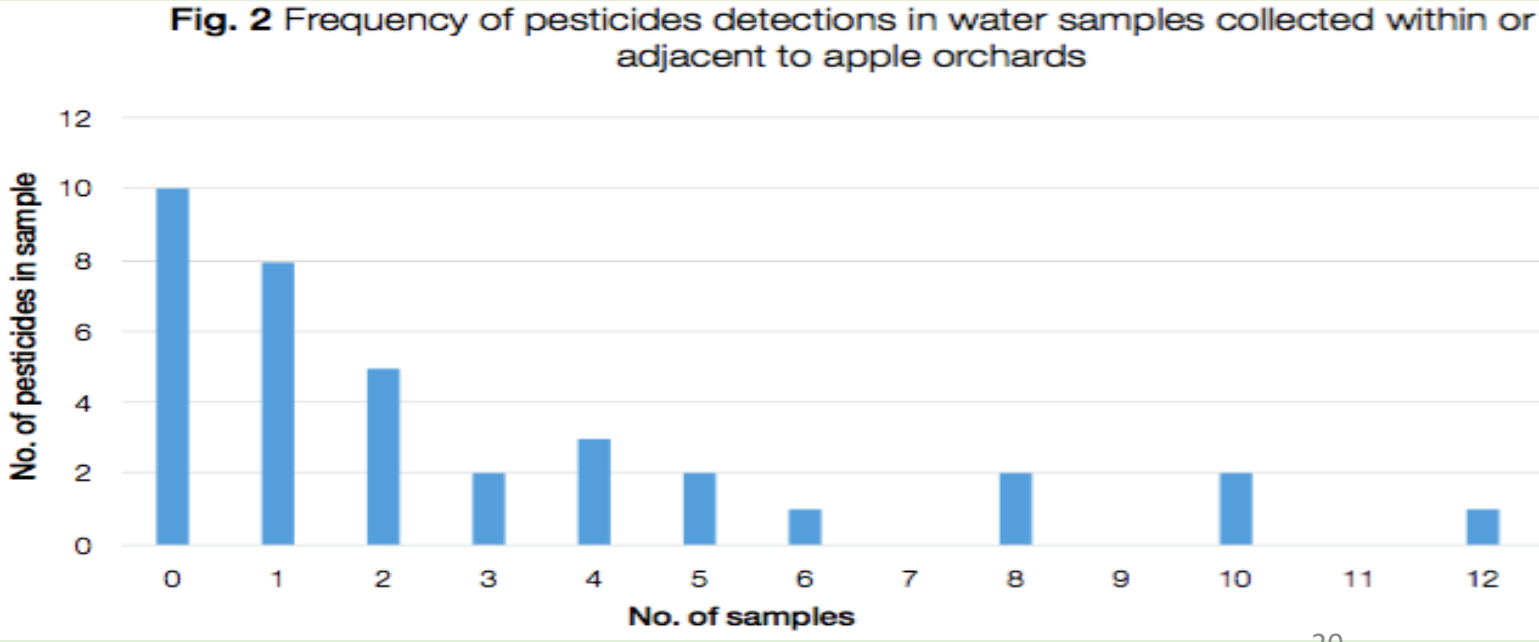
Pesticides in European apples, 2015

Table 1: Number of residues found in samples from each country

			Number of residues found in conventionally grown apples									
	No. of samples	Organic samples (without residues)	0	1	2	3	4	5	6	7	8	Mode of residues / mean number of residues found per conventional sample for each specified country
Austria	10	1	1	1	1	3	2	1	0	0	0	3 / 2.8
Belgium	4	1	0	0	1	0	2	0	0	0	0	4 / 3.3
Bulgaria	5	2	0	0	2	0	0	0	0	0	1	2 / 4.0
Switzerland	8	2	1	3	0	1	0	1	0	0	0	1 / 1.8
Germany	39	6	4	12	7	5	3	1	0	1	0	1 / 2.0
France	13	1	6	3	0	0	3	0	0	0	0	0 / 1.3
Italy	10	1	1	5	2	1	0	0	0	0	0	1 / 1.3
Nether-lands	5	0	0	0	1	2	1	1	0	0	0	3 / 3.4
Poland	10	0	0	3	2	2	1	2	0	0	0	1 / 2.7
Slovakia	8	0	0	3	2	1	0	2	0	0	0	1 / 2.5
Spain	14	3	0	1	0	3	3	1	1	2	0	3 / 4.3

Pesticides in European apple orchards, 2015
…numbers of residues





Pesticides in European apple orchards, 2015
…types of residues

Pesticide	Class/ type	Frequency of detection in soil samples		Countries in which detected (number of samples) [concentration range in mg/kg]
		No. of samples	% samples	
2,4-D	H	1	2	Switzerland (1) [0.084]
Boscalid	F	19	38	Austria (1) [0.14], Belgium (3) [1.4-3.6], France (4) [0.28-0.72], Greece (1) [0.073], Italy (2) [0.16-3.1], Netherlands (3) [0.12-0.25], Poland (3) [0.11-0.31], Slovakia (2) [0.11-0.35].
Carbendazim	F	4	8	Belgium (1) [0.11], Germany (2) [0.072-0.13], Italy (1) [0.57]
Chlorantraniliprole	I	9	18	Belgium (3) [0.083-0.14], France (2) [0.05-0.057], Germany (2) [0.1-0.16], Greece (1) [0.089], Italy (1) [0.062]
Chlorpyrifos-ethyl	I (op)	10	20	Austria (1) [0.077], France (4) [0.02-0.26], Italy (1) [2.1], Switzerland (4) [0.03-0.21]
Cyprodinil	F	4	8	Belgium (1) [0.11], France (1) [0.23], Germany (2) [0.077-0.099]
DDT (as DDD and DDE)	I	13	26	France (2) [0.015-0.023], Germany (2) [0.083-0.184], Hungary (3) [0.015-0.11], Netherlands (4) [0.036-0.4], Poland (2) [0.019-0.092]
Deltamethrin	I	1	2	Italy (1) [0.07]
Dieldrin	I	1	2	Greece (1) [0.072]
Difenoconazole	I	8	16	Belgium (2) [0.2-0.26], France (2) [0.073-0.096], Italy (1) [0.23], Poland (1) [0.095], Switzerland (2) [0.083-0.14]
Diflufenican	H	2	4	Belgium (2) [0.36-0.53]
Endosulfan (as Endosulfan sulphate)	I	3	6	Austria (1) [0.076], Italy (1) [0.03], Switzerland (1) [0.03]
Endrin	I	1	2	Austria (1) [0.04]
Etofenprox	I	1	2	Italy (1) [0.29]
Fenbuconazol	F	1	2	France (1) [0.061]
Fenhexamid	F	1	2	Italy (1) [0.18]
Fludioxonil	F	6	12	France (4) [0.069-0.33], Germany (1) [0.07], Italy (1) [0.069]
Fluquinconazole	F	2	4	Austria (1) [0.11], Germany (1) [0.03]
Flusilazol	F	2	4	Poland (2) [0.05-0.23]
Imidacloprid	I (neo)	1	2	Italy (1) [0.081]
Indoxacarb	I	4	8	Belgium (2) [0.018-0.061], Italy (1) [0.32], Slovakia (1) [0.02]
Iprodione	F	1	2	Italy (1) [1.8]
Linuron	H	1	2	Belgium (1) [0.06]
Methoxyfenozide	I	3	6	Germany (2) [0.062-0.091], Poland (1) [0.18]
Mecoprop (MCPP)	H	1	2	Switzerland (1) [0.098]
Myclobutanil	F	3	6	Belgium (2) [0.018-0.1], Switzerland (1) [0.023]
Oxadiazon	H	1	2	France (1) [0.041]
Oxyfluorfen	H	4	8	France (2) [0.035-0.1], Italy (2) [0.055-0.21]
Penconazole	F	7	14	Belgium (2) [0.082-0.12], Germany (2) [0.05-0.11], Italy (1) [0.15], Switzerland (2) [0.053-0.1]
Pendimethalin	H	3	6	Austria (1) [0.25], Belgium (1) [0.13], France (1) [0.16]
Pirimicarb	I	3	6	Belgium (1) [0.076], Germany (1) [0.052], Italy (1) [0.15]
Pyraclostrobin	F	3	6	Belgium (2) [0.1-0.16], Italy (1) [0.19]
tau-Fluvalinate	I, Ar	3	6	France (3) [0.018-0.047]
Tebuconazole	F, P	8	16	Germany (2) [0.075-0.077], Hungary (5) [0.066-0.079], Italy (1) [2.2]
Tetraconazole	F	2	4	France (1) [0.087], Hungary (1) [0.064]
Thiabendazole	F	1	2	Belgium (1) [0.12]
Triadimenol	F	1	2	Belgium (1) [0.21]

Key
Al = algicide, Ar = acaricide, F = fungicide, H = herbicide, I = insecticide, M = microbiocide, P = plant growth regulator, op = organophosphate, neo = neonicintinoid

Pesticides in pollen & beebread from European hives, 2013

Country	Sampling period, 2013	Number of samples	Key pesticides (banned neonicotinoids and other frequently encountered pesticides*) (Number of samples in which found) [Contentration range in µg/kg]
Austria	May	3	Clothianidin (1) [4.7], Thiacloprid (1) [24], Tebuconazole (1) [30]
France	Apr-Sep	12	Boscalid (2) [48-269], Folpet (1) [11], Tebuconazole (1) [159], Thiophanate-methyl (1) [24]
Germany	May-Jun	15	Thiacloprid (8) [10-250], Amitraz (incl. metabolites) (1) [11], Azoxystrobin (2) [30-69], Boscalid (5) [12-144], Cyprodinil (2) [454-590], Fenhexamid (1) [2550], Spiroxamine (1) [10], Thiophanate-methyl (1) [17], Trifloxystrobin (2) [26-1104]
Greece	Jun-Jul	10	Amitraz (2) [20-33], Chlorpyrifos-ethyl (1) [360]
Hungary	May-Jul	7	Thiacloprid (3) [22-33], Amitraz (incl. metabolites) (4) [13-46], Boscalid (2) [18-57], Chlorpyrifos-ethyl (1) [123], Fenhexamid (1) [13], Folpet (1) [97]
Italy	May-Jul	12	Imidacloprid (2) [1.7-11], Chlorpyrifos-ethyl (3) [10-562], Boscalid (3) [13-43], Cyprodinil (2) [22-146], Dimethomorph (11) [20-2045], Fenhexamid (6) [11-43], Folpet (6) [10-1316], Iprovalicarb (7) [11-320], Metalaxyl/Metalaxyl-M (6) [12-454], Spiroxamine (7) [12-83], Tebuconazole (3) [22-296], Thiophanate-methyl (1) [29], Trifloxystrobin (7) [22-220]
Luxembourg	May-Jun	5	No pesticides detected
Poland	May-Jun	7	Thiacloprid (1) [147], Chlorpyrifos-ethyl (6) [10-119], Azoxystrobin (3) [17-22], Tebuconazole (1) [16], Thiophanate-methyl (2) [10-68]
Romania	Jun-Aug	10	Azoxystrobin (1) [18], Fenhexamid (1) [13], Folpet (1) [51], Thiophanate-methyl (2) [27-93]
Spain	Jul-Aug	14	Imidacloprid (4) [7.6-148.5], Chlorpyrifos-ethyl (5) [11-705]
Sweden	Jul	2	Clothianidin (1) [1.8], Boscalid (2) [147-1081]
Switzerland	Apr-Sep	10	Thiacloprid (1) [31], Cyprodinil (2) [91-10169], Thiophanate-methyl (1) [21]

- 17 pesticides (14 fungicides & 3 insecticides/acaricides) in pollen near vineyards in Valle S Matteo, Italy (2013)
- 7 pesticides (6 insecticides/acaricides & 1 fungicide) in beebread from Gilena, Andalucia (stored from 2012)

Higher Tier Risk Assessment for PPP: Field relevance or spurious confidence?

- Although higher tier testing may appear to yield greater rigour and relevance, it also leads to less humility and precaution
- Gaps in understanding and assessment may be less explicitly acknowledged
- Potential for combination effects in mixtures will remain particularly hard to account for, especially in higher tier testing
  - e.g. taking the 17 pesticides in one single pollen sample, if tested only in pairs, would already require 136 different combinations…in groups of 3, this rises to 680 combinations

Higher complexity in PPP risk assessment: to accept or avoid?

DO ASSUME:

- that the use of pesticides in the field will lead to exposure (to non-target species and, possibly, humans)
- that wildlife and humans will experience exposure to pesticides as mixtures of active substances (and of other ingredients)

DON'T ASSUME:

- that under higher tier testing and assessment, all bases will be covered
- that there will be no surprises

Combination effects of chemicals: some ways forward?

- Conceptual frameworks and decision trees may help guide assessment of combination effects in relatively simple mixtures of chemicals, but may rapidly reach limits
- EuroMIX initiative may yield greater insight for ‘key’ mixtures, but not clear yet how those will be selected, nor how far this will lead to robust tools for general application (nor extent of animal testing)
- Reliable assessment of combination effects of chemicals in complex mixtures (which are what are routinely encountered in the real world) will remain extremely difficult, if not impossible

Combination effects of chemicals: some ways forward?

- Need far more focus upstream on reducing exposures to such mixtures of chemicals through actions at source:
  - Avoid use of known hazardous substances wherever possible (including through substitution with safer alternatives)
  - Simplify the formulation of products as far as possible (and control & monitor composition)
  - Minimize the generation of wastes and their complexity (including by auditing and control of inputs to waste streams)
- For existing/legacy pollution, take whatever practical steps are available to minimise exposure, to contain contaminated materials and/or remediate contaminated sites