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NoMiracle Newsletter No. 14

NOvel Methods for Integrated Risk Assessment of CumuLative stressors in Europe

Editorial

By Hans Løkke- NoMiracle project co-ordinator

Aarhus University.
Photo: Lars Kruse/AU-Foto.

This September the sixth and final NoMiracle meeting will be held in Aarhus, Denmark. The meeting is a conference with parallel sessions, open for posters and oral presentations from scientists, who deal with multiple stressors and any issues related to the main topics of NoMiracle. In relation to parallel research, we are looking forward to present the major findings of the project and to discuss the outcome with regulators and other stakeholders, looking for new ways to solve complex problems. The programme outline and the first call for presentations are found in this issue.

In the final session of the Aarhus Conference, the options available to cope with multiple stressors will be discussed together with limitations which may be inherent in current statutory requirements. The ambition of the NoMiracle Consortium is that as many as possible of the methods developed will be applied in near future. However, facing scientific, economic or political barriers, some of the methods may require more research or the application of the methods may fall beyond REACH.

In this issue of the Newsletter I also have the pleasure to present the results from a very productive year of NoMiracle. For 2008 the three overarching priorities of the Work Programme were to analyse data from the experimental work, to make the set-up of two project master cases on 'Cumulative assessment of agricultural pesticide application', and 'Human health risk-potential of inhalative and ingestive stressors', and finally the development of an advanced concept for risk assessment.

Moreover I also want to draw the attention to the British Toxicology Society Early Career Investigator Award for 2008 presented to a NoMiracle scientist working on meta-analyses of toxicokinetic variability in humans. Congratulations to Dr. Jean Lou Dorne!

In this issue

First call for papers

Mark your calendar for the conference:

"Multiple Stressors – Novel Methods for Integrated Risk Assessment"

28th–30th September 2009 in Aarhus, Denmark

This is the **first call** for papers to be presented at the 6th open NoMiracle meeting. The conference is open for contributions in combination with presentation of the main results from the FP6 Project NoMiracle.

The Scientific Committee is putting together a strong programme including plenary keynote presentations, two parallel platform sessions and a poster session, related to multiple stressors to man and environment. More details are found in the draft programme. To augment the existing programme, papers will be accepted in the areas of:

1. integration of environmental and human risk assessment
2. assessment of chemical mixtures and combinations of chemicals and natural stressors,
3. understanding complex exposure scenarios,
4. understanding the cognitive and knowledge-related, social and contextual aspects of integrated risk assessment.

Papers that focus on the development of advanced methods in areas such as tracking the fate of chemicals, the effects of chemical mixtures and combinations of natural stressors and chemicals, toxicokinetics, toxicogenomics, modelling, social science and data handling will be particularly welcome. This applies especially where such methods have potential for improving environment and human health by reducing the risk from chemical exposure.

We are requesting abstracts for platform or poster presentations at the conference. We are particularly grateful for presentations from outside NoMiracle to compliment the work that will be presented from the project. Primary research, case study, monitoring programme data, or policy-based papers related to the programme as shown next page are welcomed. Based on the accepted presentations the final programme will be worked out.

Abstract deadline: 1st June 2009

Please find the abstract form and updated information at:
http:// nomiracle.jrc.ec.europa.au

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Dr. Jürg Oliver Straub
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Draft programme for NoMiracle conference

Multiple Stressors – Novel Methods for Integrated Risk Assessment
Aarhus, 28th–30th September 2009

Day 1	**Monday 28 September 2009**	
9:00 – 9:10	Welcome address: Aarhus University Rector Lauritz Holm-Nielsen	
9:10 – 9:30	Introduction: NoMiracle Coordinator Hans Løkke	
9:30 – 10:00	Multiple stressors I	
10:00 – 10:30	Coffee & Tea Break	
PLENARY SESSION		
10:30 – 12:00	Metabolism, Variability and Risk Assessment	
	Multiple stressors II	
12:00 – 12:30	Discussion	
12:30 – 14:00	Lunch	
PARALLEL SESSIONS		
14:00 – 15:30	**1.1** Combined effects of natural stressors and chemicals	**2.1** Matrix-compound interactions
15:30 – 16:00	Coffee & Tea Break	
16:00 – 17:30	**1.2** Metabolic fate	**2.2** New concepts and techniques for probabilistic risk assessment
19:00	Conference Dinner	
Day 2	**Tuesday 29 September 2009**	
PARALLEL SESSIONS		
9:00 – 10:00	**1.3** Toxicokinetics – uptake and interactive effects	**2.3** Explicit modelling of exposure and risk in space and time
10:00 – 10:30	Coffee & Tea Break	
10:30 – 12:30	**1.4** Patterns and trends in mixture toxicology	**2.4** Chemical measures of bioavailability
12:30 – 13:30	Lunch	
13:30 – 15:00	**1.5** Mixture effect description and prediction	**2.5** Integration of environment and human health
15:00 – 15:30	Coffee & Tea Break	
POSTER SESSION		
15:30 – 16:15	Short platform presentation of posters in parallel sessions	
16:15 – 17:30	Posters	
Day 3	**Wednesday 30 September 2009**	
PARALLEL SESSIONS		
9:00 – 10:10	**1.6** Role of chemicals in immunotoxicity and allergenicity	**2.6** Risk presentation and visualisation
10:10 – 10:30	Coffee & Tea Break	
10:30 – 12:30	**1.7** Mechanistic toxicology	**2.7** Region-specific environmental fate
12:30 – 13:30	Lunch	
PLENARY SESSION		
13:30 – 14:30	Dealing with uncertainty and ambiguity in risk management	
14:30 – 15:00	New strategies for testing and risk assessment	
15:00 – 16:00	General discussion on the topics of Days 1–3	
16:00	Closure of the conference	

NoMiracle **Main Results 2008**

Concerning the risks from multiple stressors, NoMiracle researchers revealed significant differences in the perception of risks between lay persons and researchers. During 2008, the framework for investigating mixture effects has elucidated the key role of toxicokinetics and toxicodynamics to explain interactive effects caused by chemical mixtures. Among other findings, Chemical Activity was confirmed as a promising key parameter for bioavailability, largely eliminating cross species differences in response to narcotic acting chemicals. Further progress has been achieved on Dynamic Energy Budget modelling as an efficient tool for assessment of mixture toxicity.

How are Multiple Risks Perceived?

During the reporting period NoMiracle has explored how multiple chemicals and cumulative stressors are perceived among the public as a part of everyday life. In the examined cases lay persons generally worried relatively little about cumulative risks in contrast with the results of the extensive NoMiracle survey on the concepts and views held by experts, key EU regulators and EU-level stakeholders on integrated treatment of cumulative risks from multiple stressors. Lay persons experience cumulative risks all the time; it is a part of everyday life whereas scientists may worry about cumulative risks when their conventional studies focus on single stressors. The uncomfortable thought arises that those exposure concentrations of single stressors, which are considered safe by conventional risk assessment techniques may no longer be safe when they are considered in a "cumulative exposure context". The findings of NoMiracle suggest that there is a need to reflect more deeply on the relationship between the development and use of risk assessment techniques for regulatory purposes and the distribution of risk information in society. The work on risk perception is in the process of being published as articles in peer-reviewed journals.

New framework for assessing the risk of chemical mixtures

Concerning the risk assessment of multiple stressors, NoMiracle has established a large database resource that holds the summary results of all mixture toxicity data collected in the effect assessment phases of NoMiracle. These data will be used in the final phase of the project to assess the frequency and magnitude of interaction within mixtures. This work is one of the key outputs of NoMiracle, as it will allow us to estimate the uncertainty associated with predictions of the toxicity of mixtures made using the CA (Concentration Addition) and IA (Independent Action) models. This can allow these models to be used in a probabilistic context and can also be used to assess whether current safety factors applied in the derivation of predicted no effect concentrations provide a reasonable protection against interactive effects in mixtures of stressors.

To understand mixture and multiple stressor effects in a more holistic way that goes beyond mere description, a theoretical framework has been developed across NoMiracle that can be used to provide a more complete understanding of interactions in mixtures. Development of this framework has helped us to identify the possible location at which interactions are most likely to occur. Four possible sites of interaction were identified.

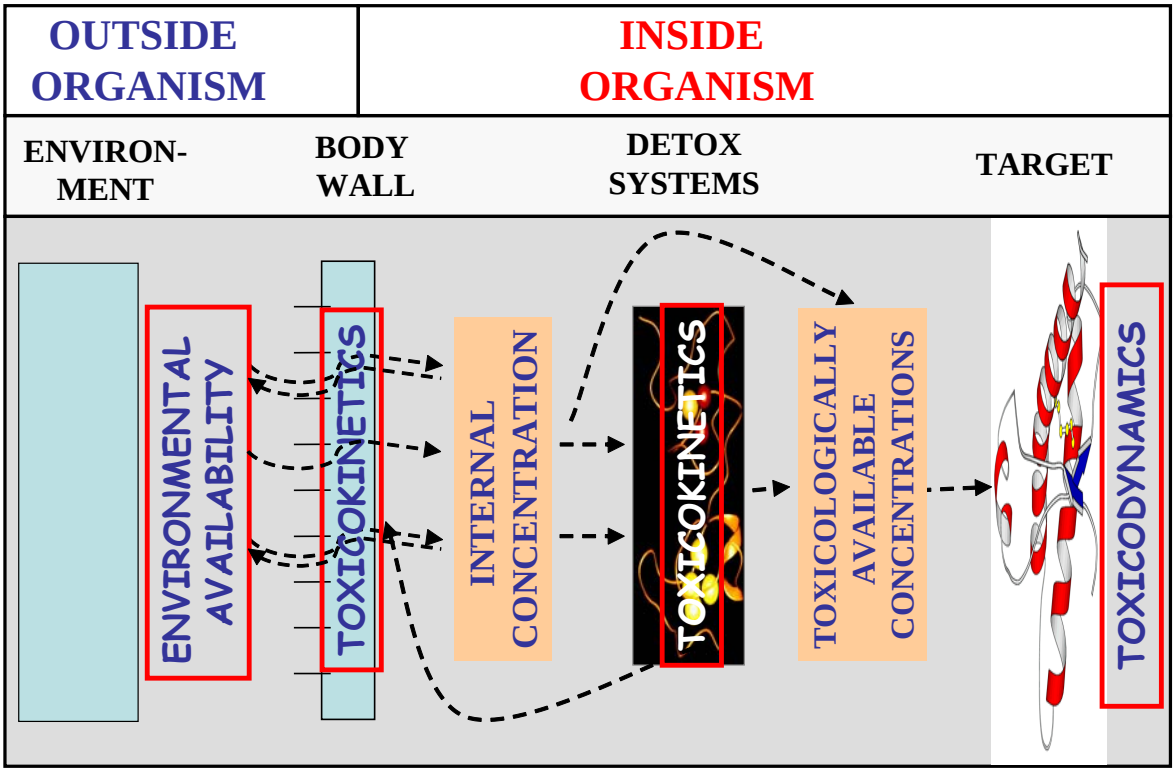
1. Interactions in the environmental media that change the environmental availability of one or more chemicals in the mixture.
2. Interactions occurring at site of uptake and/or elimination of the chemical from the organisms that result in modulation of the total accumulated internal concentration of one of more of the chemicals in a mixture.
3. Interactions resulting in a change in the rate of detoxification/compartmentalization of one or more chemicals in a mixture.

4. Interaction at the target site that affect the binding of one or more chemicals in a mixture to a receptor through which a toxic effect is mediated.

Identification of these sites of potential interaction provides a basis upon which to select methods that may be used to understand the mechanism of action and interaction of chemicals. A graphic representation of the framework is given in the figure below.

Framework used to investigate mixture effects focussing on the key role of toxicokinetics and toxicodynamics to explain interactive effects in mixtures. Red border indicated potential points of interaction between chemicals in mixtures.

A second aspect to identifying mixture effects is to gain a better understanding of the interaction of the chemicals at the target site and the range of resulting toxic effects that take place. This work is being pursued in some detail at the molecular level using a range of toxicogenomic tools. Thanks to an extensive period of method development that has built substantially on work in forerunner projects, NoMiracle scientists have been able to conduct one of the most extensive cross-species studies on the molecular basis of toxic chemical effects yet conducted. This work provides a unique data resource that can be used to investigate the similarity in the mode of action of chemicals for 1) similar chemicals (i.e. pesticides from the same class) in the same species; 2) chemicals with distinct modes of action in the same species and, 3) identical chemicals in different species. This information will be a valuable contribution to the



debate concerning the definition of modes of action for use in mixture effect prediction, the "read across" value of biomarkers between humans and environmental species and the mechanistic similarities of chemical effect in similar and diverse taxa. The read across use of surrogate species for toxicity analysis is also important in the context of the development of tools for human biomonitoring and also in the development of alternative methods to toxicity testing using non-vertebrate species.

Cumulative effects of chemicals and natural stressors can be described by current models

The research has shown that natural stressors (e.g. associated effects of climate change) can be described using models that are currently used for chemicals. This means that combined effects of chemicals and natural stressors can be estimated by mathematical models based on data. The approach further allows safety factors and uncertainty to be estimated for combined stressors. The results are now in the process of publication in a number of articles in peer-reviewed journals.

New paradigm: Identification of chemical compounds with specific mode of action

Within the overall framework, NoMiracle produces novel methods for estimating the impact of cumulative stressors. By introducing the unitless chemical activity as the parameter for exposure and bioavailability, narcosis or baseline toxicity was observed to be exerted at a rather constant level of chemical activity (0.01 - 0.1). This was the case for various non-polar chemicals, for different exposure routes (water, air, direct contact) and across species (e.g. algae, worms, tadpoles, mice - and probably man). Toxicities at chemical activities well below 0.01 may indicate specific mode of toxic action. This finding neglects the role of the toxicokinetics, meaning that the statement more or less is limited to the equilibrium partitioning situation. The findings implicate that future testing needs to be directed towards the identification of compounds with specific modes of action, and pointing out those.

In parallel to the mixture toxicity work, the workpackages on "Toxicokinetic modelling" and "New concepts and techniques for probabilistic risk assessment" developed a new concept for assessing mixture toxicity based on the Dynamic Energy Budget theory (DEB). The combined effect of chemicals with narcotic action can be estimated quite precisely by use of very few data on one component of the mixture. This means that the finding also indicates that the testing effort can be directed towards a search for specific action of single compounds, and in the case of mixtures, for modelling the likelihood of deviations from expected toxic action using general models. Further, it is now possible to interpret sublethal effects of mixtures within one consistent framework: Biological mechanisms can be classified under certain interactions, instead of regarding interactions as some statistical deviation from some chosen standard. This finding may induce a shift of paradigm in the assessment of mixture effects. Traditionally, toxicological testing uses one fixed exposure time. The new method requires measuring at different exposure times with less replicates.

New methods for assessing the impact of actual chemical cocktails

The conventional stressor-oriented approach, following the fate of the stressor through the environment and through time is not very appropriate to describe cumulative exposure of actual chemical cocktails in the urban environment or in ecosystems. NoMiracle has developed novel tools or experimental methods in the fields of multimedia modelling and human and wildlife random-walk modelling, as well as in the application of the ecological and spatially explicit cumulative exposure model Eco-SpaCE. The work on indoor and urban models for exposure to cumulative

stressors has been extended to include modelling of meteorological effects. NoMiracle has made it clear that "vulnerability" is a crucial issue in cumulative risk assessment. It is not only the (combination of) stressors that determine the risk. It is the life-history strategy of exposed organism (e.g. slow and extensive metabolisers), its exposure history, its life style, its movement pattern, its activity pattern etc that determine the vulnerability. Instruments to identify "vulnerable" individuals/groups play a key role in accurate assessment of cumulative risks. The research has also revealed a need for receptor-oriented approaches focussing on the exposed organism when describing the spatio-temporal pattern of exposure to multiple stressors. The translation of these spatio-temporal exposure profiles into effects is currently problematic, because most effect studies apply homogeneous exposure regimes. Cumulative risk is not just mixtures, i.e. simultaneous exposure to constant concentrations of multiple stressors, but also sequential exposure to multiple stressors at varying concentration. There is still a lot of work to be done in this area. The approaches developed in NoMiracle are promising, but NoMiracle will not solve the whole puzzle of cumulative risk assessment.

International workshops

NoMiracle held two open workshops during the last year. The Workshop in Leipzig "Novel Methods for Assessing Chemical Exposure" 1-2nd April 2008 was a forum for presenting and discussing the novel and often "paradigm-shifting" methods for estimating exposure developed in the project. These novel methods could become commonplace in exposure assessment later in the 21st century. The workshop invited a broad audience of stakeholders from companies, NGOs, regulators, EU representatives, academia (especially young researchers and PhD students) and project partners from related EU projects. The Workshop report is published in NoMiracle Newsletter No 12 from May 2008.

The following workshop "Integrated Assessment of Environmental and Human Health" in Frankfurt 8-9th September 2008 addressed how data on the effects of chemicals on humans and the environment can be used to provide a more holistic assessment of the consequences of exposure to single and multiple contaminants. The area of uncertainty analyses was placed at the heart of many talks, helping to elucidate the relative share of the human and non-human components in the overall uncertainty and the options for integration across receptors and sectors. The workshop made it clear that there is a lack of legislation and regulation to tackle cumulative risks. Some tools will be delivered from NoMiracle, however, also regulation (laws/guidelines/institutions) are needed that address cumulative risks in an appropriate way. The Workshop report is published in NoMiracle Newsletter No 13 from January 2009.

Demonstration of the Novel Methods in Master Cases

During the third and fourth year two master cases have been developed:

- Cumulative assessment of agricultural pesticide application
- Human health risk-potential of inhalative and ingestive stressors

The aim is to demonstrate the utility of the new knowledge and novel methods developed in NoMiracle to those involved or interested in EU risk assessment practice. The application of the new approaches will be compared with the existing risk assessment techniques. A series of training events for stakeholders will be conducted in the last year of the project.

Award to NoMiracle Scientist

By Claire Mays

The British Toxicology Society (BTS) has presented Dr. Jean Lou Dorne with the **BTS Early Career Investigator Award for 2009**. Dr. Dorne received the award at the BTS Annual Congress at the University of Warwick, 22nd – 25th March. Dr. Jean Lou Dorne worked at the University of Southampton for the NoMiracle project on the development of meta-analyses of quantifying human variability in toxicokinetics for mixtures. His work is important to better quantify, characterise and reduce uncertainty in current risk assessment methodologies by improving the scientific basis for the derivation of uncertainty factors. Dr. Dorne is now working for the European Food Safety Authority (EFSA) in Parma, Italy, and is finalising manuscripts for NoMiracle related to the toxicokinetics of mixtures. At the NoMiracle Conference in Aarhus, which is to be held 28th – 30th September, 2009, he will present his major findings together with EFSA's activities on mixtures.

1) Jean-Lou, congratulations! Is this your first major award?

It is the second one. I received the young scientist award at the conference of the European Societies of Toxicology (EUROTOX) in 2002 in Budapest for my PhD work on pathway-related uncertainty factors and their use in risk assessment.

2) What makes you particularly happy about this award?

What makes me particularly happy about this award is that all the work has been carried out in the UK and that it is a recognition of the British Toxicology Society (BTS).

3) Which part of your work is being recognized? Is it NoMiracle work? What are the applications or implications of the work?

Again, the BTS award is a recognition of a contribution to toxicological sciences as a whole, which covers all the work we have done over the last ten years and also includes the research conducted for NoMiracle.

In chemical risk assessment for non-genotoxic compounds, we have been using a 100-fold uncertainty factor applied to a non-toxic dose in laboratory animals (No-observed adverse effect level) to derive a safe level for these compounds (health-based guidance value) that would not have any adverse effects when consumed over a life time, i.e. acceptable or tolerable daily intake. The 100-fold factor covers interspecies differences (10-fold) and human variability, and both 10-fold factors have been subdivided to cover the elimination aspect (toxicokinetics) and the toxicity aspect (toxicodynamics) and allow for chemical specific data to replace these default values. For humans, both aspects have been subdivided to $10^{0.5}$ (3.16).

This type of work contributes to move towards more science-based uncertainty factors for risk assessment that is taking into account human variability in toxicokinetics-differences between human populations (polymorphisms, inter-ethnic differences, children, neonates, the elderly) in the way they eliminate chemicals. Fourteen major pathways of metabolism and renal elimination were analysed for the different populations available from the therapeutic drug database using meta-analysis techniques to then derive pathway-related variability and pathway-related

uncertainty factors. These can replace the standard uncertainty factors of 3.16 for the toxicokinetic aspect when one knows the metabolic route of the compound.

Data for binary mixtures were also analysed to take into account the effects that an inhibitor or an inducer would have on the elimination of a compound. This kind of analysis has implications for risk assessment and can inform risk assessors of potential mixtures and metabolic pathways that would be of priority because of the potential effects and large variability between populations.

Overall, we do try to identify the metabolic pathway involved in the metabolism of a particular chemical and move towards more quantitative approaches using human data from the therapeutic database. Monte Carlo models based on such quantitative data have also been developed to predict human variability and uncertainty factors for chemicals metabolized by multiple pathways.

4) What precisely did you present at the British Toxicology Society Annual Conference in late March, when you accepted the award? What was the response?

The presentation focused on the history of chemical risk assessment and the evolution of uncertainty factors, then the development of pathway-related uncertainty factors and the development of predictive Monte Carlo models for chemicals metabolized by multiple pathways using pathway-specific variability.

Finally, NoMiracle was also presented, including the structure, the collaboration with Dr. Ad Ragas (Niemigen University) on the harmonisation of ecological and human risk assessment using mechanistic descriptors (toxicokinetics, toxicodynamics) and, finally, the analysis of toxicokinetic data for mixtures in humans, again with the implications for risk assessment.

I thought the response was positive, and the presentation is currently being written up as a paper for toxicology *(Toxicology).*

5) An "early career investigator" award is a sign of confidence that you will go even farther. Which directions do you see your work taking in the future?

This work is still ongoing, and we are now collaborating with colleagues to implement refined statistical methods (Bayesian) in the derivation of such uncertainty factors and applying meta-analysis methods to the risk assessment of food contaminants. An example of this is the work we have done here at the European Food Safety Authority for cadmium using biomarkers of renal effects to derive a safe level of exposure for humans between the assessment methodology unit (Dr. Billy Amzal and Dr. Eugen Christoph) and the unit on food contaminants (where I work).

In parallel, a whole book on human variability in toxicokinetics and the implications for risk assessment is currently under preparation for Wiley's inter-science in collaboration with Dr. Billy Amzal for the modelling and statistical aspects, and Dr. Sami Haddad for the modelling and mixture aspect. I will contribute on the metabolism and uncertainty factor aspects.

6) Any advice for other "early career investigators"?

Put your heart into it, enjoy it. Keep at it even when difficulties arise with lots of patience and a positive attitude.

Dorota Jarosinska
European Environment Agency representative in the NoMiracle Advisory Board.

New member of NoMiracle Advisory Board

Dorota Jarosinska, MD, PhD graduated from the Silesian Medical University, Katowice, Poland. She is a specialist in public health, working mainly in environmental medicine and environmental health. She participated in a year long postgraduate course on 'Advanced Environmental Sanitation' at IHE, Delft, the Netherlands, and in a two-year long training in environmental health for physicians at the Institute of Occupational Medicine and Environmental Health in Sosnowiec, Poland. She was a Fulbright scholar at the National Institute of Environmental Health Sciences, RTP, NC, USA. Professional experiences cover the clinical practice of environmental medicine, projects on prevention of environmental health hazards in adults and children, and research, focusing on human biomonitoring of heavy metals. For several years she cooperated with the WHO, European Centre for Health and Environment on projects on environment and health information system based on indicators. Since 2005, she has been working for the European Environment Agency in Copenhagen, Denmark, as a seconded national expert in environment and health. She authored several scientific papers and contributed to the reports by EEA, WHO, and UNEP.

NoMiracle co-ordination

Visit NoMiracle and subscribe to the Newsletter at: http://nomiracle.jrc.ec.europa.eu

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