

AOP

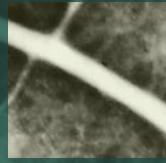
THE TROJAN HORSE
FOR INDUSTRY LOBBY TOOLS?



**Pesticide
Action
Network**
Europe (2016)

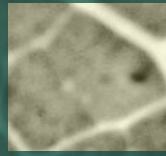
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FUTURES

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Summary



AOP, THE ADVERSE OUTCOME PATHWAY, is a very interesting research topic. With AOP scientists try to find out HOW adverse effects develop in the body. AOP could be used in some future for screening of chemicals with unknown toxicity if it matures and shows good predictability of adverse effects. Use as a final decision-taking tool in chemical risk assessment is an illusion for the foreseeable future because AOP has an unknown level of prediction and cannot guarantee the high level of protection that is required by EU law. Current AOPs also fail to take into account the effects of mixtures of chemicals. But is this illusion not the hidden agenda of chemical industry? Getting rid of the expensive animal testing and substituting it by low-cost AOP? And even questioning any (undesired) outcome of animal testing? This could be inferred from the massive efforts industry is doing to help designing AOP. Millions of taxpayers' money are derived from the EU research programs to support these industry initiatives. Since AOP will be used to regulate chemicals that the general public is exposed to, one would expect that at least an independent body to be at the steering wheel of AOP. But this is not the case. Government officials are closely operating with industry, without the presence of other public society stakeholders. Industry is writing its own rules.

Those government experts involved in developing AOP in their enthusiasm easily forget that the situation at implementation level is totally different than the scientific atmosphere during the development phase. At the implementation level of Brussels risk assessment scientific discussions are substituted by political dealing and wheeling and power play. Anything goes and science doesn't count that much anymore. This is the more the case given the unknown level of predictability of AOPs that allows for much speculation and assumptions, the so-called "expert judgement". A massive misuse of AOP can be foreseen if a chemical company fights to get their chemical on the market, no matter how. Currently the first examples of this misuse can be observed already in the initiative of the fragrance industry to predict adverse effects solely based on assumed similar chemicals of known toxicity. Also in the EU approval of pesticides the first examples can be observed; Health DG SANTE even allows overruling of adverse outcomes observed in animal testing. Priority setting and assisting on filling data gaps for unknown chemicals should be the objective of AOP, not overruling adverse effects in animal experiments. European Commission has to act to make sure AOP is only used as a first screening of unknown chemicals and stop the use in risk assessment and any other misuse.



Introduction

The political decision to ban animal testing for cosmetics in 2013 is a result of years of fighting against unnecessary testing on animals and it makes perfect sense. Why sacrifice animals for safety testing of not really essential synthetic chemical substances that are applied just on the skin? Industry will be happy to get rid of expensive animal testing requirements but still, they need to provide some kind of information on the toxicity if they like to get their synthetic chemicals approved.

CHEMICAL EXPOSURE has played an important role in the development of human health problems we see today, as well as in ecosystems' health decline and

degradation. This has become more evident with the dramatic increase of the amount of synthetic chemicals produced annually in the last decades. The various chemical regulations are gradually becoming more rigorous and demand higher chemical safety, which would mean that people and the environment get a better protection. While the hidden costs of chemicals for society sum up to many billions of Euro's on a yearly basis¹, the costs for industry in the assessment of chemicals have also increased significantly and this is what likely plays a major role in chemical industry's efforts to replace animal testing².

Industry and European Commission in 2005 set out to start a partnership³ to promote non-animal testing with the intention

1. Report on the Costs of Inaction on the Sound Management of Chemicals, UNEP, 2013
2. THRESHOLD OF TOXICOLOGICAL CONCERN (TTC), S. Barlow, ILSI Europe, 2005
3. http://ec.europa.eu/growth/sectors/chemicals/epaa/index_en.htm

to cover several animal tests. In 2008 industry (COLIPA, the cosmetics industry umbrella group) offered 25 Millions of Euros for a research program⁴ on the condition Commission would fund the same amount. This was finally agreed in 2009⁵ and the research program SEURAT⁶ started in 2011, to run till the end of 2015.

It was a FP7-program in public-private partnership style⁷.

The outcome of SEURAT was presented in a symposium⁸ in Brussels in December 2015 and Mark Cronin⁹, one of the main experts of SEURAT, highlighted three main achievements of the program:



TECHNOLOGIES

from *in silico* to *in vitro*



INTEGRATION

applying the SEURAT-1 conceptual framework through the 'Adverse Outcome Pathway' (AOP)



REALISATION

gaining international acceptance

SEURAT is now followed up by another program in 2016, EU-ToxRisk¹⁰, with similar objectives as SEURAT and again with heavy industry participation such as BASF, Cosmetics Europe, Hoffmann-La Roche, L'Oreal and Unilever, paid with 30 million EU taxpayers money. The ultimate goal is "to deliver reliable, animal-free hazard and risk assessment of chemicals".

While environmental NGOs have not been involved in this regulatory initiative (and not directly informed) so far, it is important to evaluate the process, outcome and

regulatory consequences of these programs from a public-protection point of view. This is especially the case now that the OECD launched a similar initiative¹¹ to reduce animal testing, a 'knowledge base' for AOP, a cooperation with US-EPA and EU-JRC (the same institute that was heavily involved in SEURAT), an extension of the OECD initiative in 2012 to develop a comprehensive AOP framework.

Following an "access to documents" request to EU-JRC (via DG Research) and the documents obtained, PAN Europe evaluates in the present report AOP.

4. Letter from the President of COLIPA to Gunther Verheugen, vice-President of EU Commission and responsible for Enterprise and Industry, 23-01-2008.

5. Meeting 10 March 2009 based on 'Note for the file' of Mr. Jacob, HoU, Research Directorate General.

6. www.seurat-1.eu/

7. "This FP7 Research Initiative was created through a call for proposals by the European Commission that was published in June 2009. The Cosmetics Europe industry offered to match the European Commission's funds to make a total of EUR 50 million available to try to fill current gaps in scientific knowledge and accelerate the development of non-animal test methods. The Research Initiative focuses on the complex area of repeated dose toxicity".

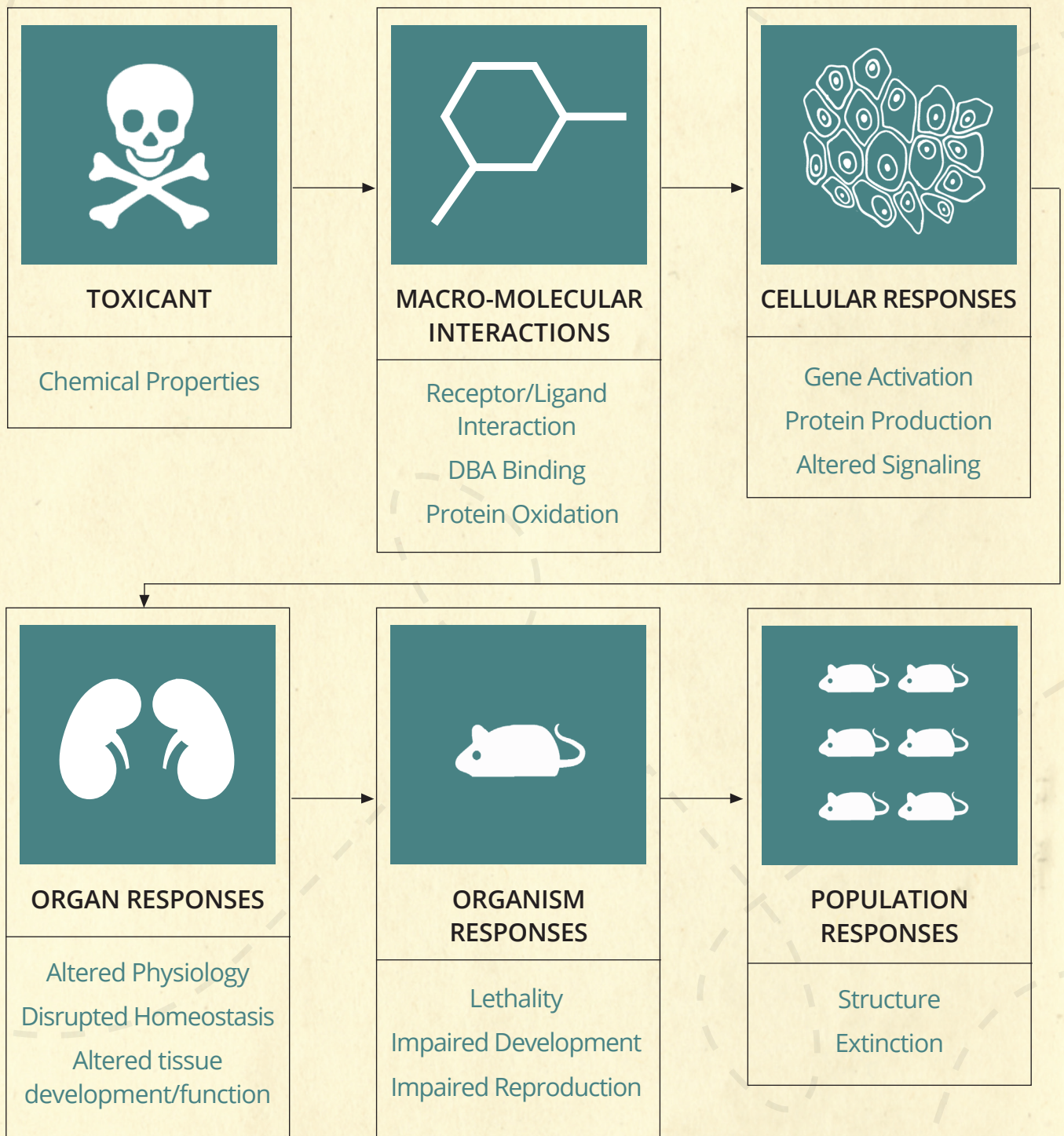
8. www.seurat-1.eu/pages/library/events/seurat-1-symposium.php

9. Professor at Liverpool John Moores University, England

10. www.eu-toxrisk.eu/

11. www.oecd.org/chemicalsafety/launch-adverse-outcome-pathways-knowledge-base.htm

Schematic representation of the Adverse Outcome Pathway (AOP) illustrated with reference to a number of pathways¹²



12. Diagram taken from:
www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

The adverse outcome pathway (AOP) is a conceptual framework originally developed to collect and produce pathway-based data to support ecotoxicology research and ultimately to be used in risk assessment¹³.

EACH AOP describes a sequential chain of causally linked events at different levels of biological organisation (molecular, biochemical, cellular, organ-level) that lead to an adverse health or ecotoxicological effect. An AOP begins with a molecular initiating event (MIE), which is defined as the interaction between a xenobiotic and a specific biomolecule, such as inhibition of an enzyme due to competitive binding of a chemical in its active site. The MIE is followed by a progression of a defined series of key events (KEs) that are measurable through *in vitro* or *in vivo* assays, necessary for the development of the toxicological outcome, and connected by key event relationships (KERs). These KEs and KERs then lead to an apical outcome that is relevant for regulatory purposes. Such outcomes may be changes in survival, development, and reproduction at the population level in ecotoxicology; or disease and organ dysfunction in human individuals and other animals.

Identifying these series of interactions that lead to an adverse effect at the organism/population level is valuable for scientific research and will contribute to our understanding of toxicity and diseases. In parallel the different AOPs might highlight the complexity of the biological systems and their interaction with chemicals. The AOP framework may serve as a “knowledge” guide in the development of tools for risk assessment to avoid placing or maintaining chemicals in the market that may cause harm to human and the environment.



13. Martin B. Phillips, Jeremy A. Leonard, Christopher M. Grulke, Daniel T. Chang, Stephen W. Edwards, Raina Brooks, Michael-Rock Goldsmith, Hisham El-Masri, and Yu-Mei Tan, 2016. A Workflow to Investigate Exposure and Pharmacokinetic Influences on High-Throughput *In Vitro* Chemical Screening Based on Adverse Outcome Pathways, *Environ Health Perspect* 124:53–60

The AOP itself is chemical-independent to allow for a general interpretation of results based on common modes of action and biological pathways. Practical application of AOPs in chemical-based risk assessment, however, will require extrapolation of an *in vitro* concentration expected to trigger an MIE to an *in vivo* biologically effective target tissue dose, which can then be used to estimate a regulatory-relevant external dose (i.e. using reverse toxicokinetics). This extrapolation cannot be made without considering exposure, the absorption, distribution, metabolism, and excretion (ADME) properties of a chemical. For now this extrapolation idea is pure speculation, much promoted by industry¹⁴, and it remains to be seen if this idea could turn into a reliable option in a far future.

AOP is a new way of risk assessment of chemicals, "US-style", promoted by US National Academy of Sciences in its publication on "Toxicity testing in the 21st century: a vision and a strategy"¹⁵. This new approach, although it may seem promising, needs to be scrutinised on its capabilities to reliably predict toxic effects of potentially dangerous chemicals in the market.

14. Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, et al (2003). Risk characterisation of chemicals in food and diet. *Food and Chemical Toxicology* 41:1211-1271

15. Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N, Knudsen T, Martin M, Padilla S, Reif D, Richard A, Rotroff D, Sipes N and Dix D (2012) Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chemical research in toxicology* 25:1287-1302.

Questions are -among others-



how accurately models can predict the reality of biological systems,



if data derived from traditional testing done on adult animals with high doses -mimicking situations that operators may get exposed to- have any value for the general public (including the vulnerable),



if AOP might be misused in the implementation phase of (traditional) risk assessment,



if AOP can guarantee the high level of protection that is required under EU law.

The AOP steps are especially useful for elucidating mechanisms of action, which is badly needed for hazard assessment of chemicals; but considering the limited knowledge we have on the development of diseases and toxicity in individuals, toxicity testing in mammals will always remain needed to meet the various legal requirements on chemicals to guarantee safety. Therefore, other motives will play a role when industry (and some regulators?) advocate their use as a full a substitute to risk assessment.



AOP, opportunities for implementing the precautionary principle

Many thousands of chemicals are on the market without prior safety testing. For the three important communication systems of the body, the nerve, immune and endocrine system, even almost no knowledge is available on toxic effects of the chemicals on the market¹⁶.

PEOPLE and the environment therefore are put at (potentially big) risks because of exposure to chemicals with unknown toxicity and to mixtures of those chemicals. The conclusion is that chemical industry has managed to maximise profits by shifting the external (health and environmental) costs to society also because politicians failed to impose full testing requirements for chemicals.

16. EEA, UNEP (1998). Chemicals in the European Environment: Low Doses, High Stakes? The EEA and UNEP Annual Message 2 on the State of

This is the case for the majority of chemicals in the regulatory EU program REACH¹⁷. If these chemicals were properly monitored (authorisation, use and waste management) and restricted from specific uses, human and the environment would be less exposed and the health costs would be far lower. The costs of human suffering and environmental damage due to these chemicals cannot be easily calculated but are massive without any doubt¹⁸. The AOP initiative to elucidate mechanisms of action could help predict and identify the most risky chemicals and restrict them.

The regulatory sector, up to now, is mainly focussed on deriving 'no effect levels' based on animal test guidelines (TGs) developed in the OECD and implemented by industry itself with GLP (Good Laboratory Practice) certified laboratories. In the testing requirements mechanistic information was generally not required¹⁹. Most mechanistic information of pesticides/chemicals is currently found in independent academic research studies, which are generally published many years after market access of the chemicals and at a time when harm has been observed.

But, very remarkably, so far, academic research is not being taken into account in regulatory decisions²⁰.

Industry was the one who has been promoting the use of mechanistic information for a long time as a substitute of animal testing and has been using it for questioning the outcome of animal testing^{21, 22}. Now industry has taken the initiative -after the animal testing ban for cosmetics took effect- to develop an approach for cosmetic chemicals based on mechanistic information. The AOP framework is their focus.

It has to be stressed that AOP is still in its infancy and not ready for regulatory use, also according to the OECD²³. The proposed use of AOPs is to predict toxicity and prioritise chemicals of concern lacking toxicity data, reducing health risks. If AOP would mature, it could be used to evaluate the many thousands of unknown chemicals as a first alert. Based on the outcome of AOP, the EU precautionary principle should be applied to ban or restrict potential hazardous chemicals from the market, while other potentially toxic chemicals will require additional testing.

17. http://ec.europa.eu/environment/chemicals/reach/reach_en.htm

18. Bellanger M, Demeneix B, Grandjean P, Zoeller RT, Trasande L (2015). Neurobehavioral Deficits, Diseases, and Associated Costs of Exposure to Endocrine-Disrupting Chemicals in the European Union. *J Clin Endocrinol Metab.* 100: 1256-1266.

19. http://ec.europa.eu/food/plant/pesticides/approval_active_substances/eu_rules/index_en.htm

20. PAN E report Missed and Dismissed

21. Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, et al (2003). Risk characterisation of chemicals in food and diet. *Food and Chemical Toxicology* 41:1211-1271

22. PAN report A Poisonous injection

23. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, 2013



Limitations of the AOP framework

OUR SOCIETY has got dependent on the use of synthetic chemicals, many of which have properties that are toxic for human and the environment. To protect humans, animal testing has been the first choice for decades now. There are several ways to reduce animal testing and the number of experiments performed by the industry but this report will focus just on AOPs. AOP, the Adverse Outcome Pathway, is now heavily pushed as the alternative framework, even though it's in an "immature" stage. By giving AOP and its "mode of action" a central place for understanding adverse effects and substituting animal testing, it must be verified that AOP is, as promised a prediction tool with a high reliability.

Verification according to OECD-standards in the AOP-production scheme²⁴ is a two-step procedure (internal, external) at the review phase on its way to endorsement. The internal review is done by 3 reviewers from the Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST). The main objective of this review is to check compliance of the AOP description with the User's Handbook²⁵. It's done independently of the reviewers' field of expertise. Based on the outcome of the internal review, the external review is started.

24. www.oecd.org/env/ehs/testing/AOP%20process_10%20June%202013.pdf.

25. USERS' HANDBOOK SUPPLEMENT TO THE GUIDANCE DOCUMENT FOR DEVELOPING AND ASSESSING AOPs, link: User's Handbook

The external review is done by experts outside EAGMST, who have scientific expertise in the hazard area/endpoint covered by the AOP. Relevant expert groups established in the context of EAGMST decide if an AOP is ready for external review. Guidelines programmes are identified and the Working Group of the National Coordinators for the Test Guidelines Programme (WNT) is requested to nominate experts based on the specific expertise needs, to update the groups or to create new groups when there's no existing group for a particular AOP. A call for participation in the external review is then sent to these groups. Not much information is available on verification and how independently this was done. Experts with industry-affiliations however are not excluded. The first external review (by 5 reviewers) was completed only recently. The role of the reviewers in the external review phase is to address the scientific/technical content of the AOP²⁶.

The comments and responses to comments from both the internal review and the external review are available publicly in the discussion pages of the AOPs that are under the list of EAGMST "Approved" on the wiki platform²⁷.

The OECD verification process therefore only looks at the compliance with the AOP-handbook and the scientific/technical contents. The verification doesn't include anything on predictability of adverse effects. This and several other limitations to AOP are discussed in the next sections.



26. OECD-docs ENV/JM/TG/M(2014)4 and ENV/JM/MONO(2005)14

27. https://aopwiki.org/wiki/index.php/Main_Page

3.1 Predictability of AOPs

Regarding the predictive value of AOPs much remains in the dark. According to the OECD²⁸, a general level of predictability of an AOP-based model is not quantifiable but AOPs could be the basis for the development and implementation of such a quantifiable model in the future. For now it is qualitative and (end)users/regulators have to decide themselves what to do with it.

Elements of the suitability for application in different regulatory contexts relies, again according to the OECD, in part on

1.

the confidence and precision with which the Key Event (KEs) can be measured,

2.

the level of confidence in the relationships between the KEs linked in an AOP-based on biological plausibility, empirical support for the Key Event Relationship (KER) and consistency of supporting data among different biological contexts - and

3.

weight of evidence for the overall hypothesised pathway, taking into account a number of additional considerations²⁹.

28. Meeting JRC/OECD/EEB/PAN Europe, February 2016

29. Perkins, E.J., Antczak, P., Burgoon, L. D., Falciani, F., Garcia-Reyero, N., Gutsell, S., Hodges, G., Kienzler, A., Knapen, D., McBride, M. and Willett, C. (2015). Adverse outcome pathways for regulatory applications: examination of four case studies with different degrees of completeness and scientific confidence. *Toxicol. Sci.* 148: 14-25

This puts a heavy burden on the regulators in the assessment of the suitability of AOP. Especially the lack of information (and data) on the reliability and predictability is a problem. This might end up in the use of AOP by “expert judgement”, and likely the “belief”, assumptions and speculations of individual experts.

The lack of information on the predictability of AOPs (and therefore any guarantee on the level of protection) questions the use of AOP in EU risk assessment of chemicals with unknown toxicity. Maybe it can add some information but acting as a decision-tool is out of the question.

This uncertainty on predictability also regards one of the elements of AOP, QSAR. Here again OECD did not describe or require a level of predictability. The actual procedure varies on a case-by-case basis (i.e., depending on the availability of similar chemicals with data), and requires the active involvement of an expert for the selection of e.g., databases and profilers (see OECD-guideline³⁰ and principles³¹). The final judgement on the validity of the predictions will be given by the Regulatory Authority, that will assess if the process followed for the specific prediction is scientifically correct, and will consider if the level of confidence / uncertainty is adequate for the use or decision-making context. The question remains if decision-making bodies will

be capable to actually make a proper assessment.

Next to questions about the (predictive) value of AOP, equally important is to look at the potential “misuse” of AOP. This misuse might not seem that obvious at a first glance but PAN Europe gets signals from everyday practice of risk assessment that points in that direction. Especially for cases when testing results are available but are deviating from AOP industry already promotes the use of AOPs and its elements as a tool, to overrule adverse outcomes. This report aims to highlight this possible misuse of the AOP framework in risk assessment.

The just finalised SEURAT-program of the industry and EU-JRC also gives the impression³² that the concerns highlighted above in relation to AOPs haven’t been addressed properly or not addressed in a robust way, some questions even haven’t been posed at all for example that of how to limit the potential misuse. Misuse is considered by OECD as “out of scope” of their activities³³. This means that AOP (or elements of AOP) in this created vacuum might very well be a main new lobby tool of industry in the political “dealing and wheeling” at the decision-time for pesticides and chemicals. In this Chapter we further discuss in detail the limitations of AOP and in Chapter 4 we will present examples of current misuse of AOP.

30. [www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2014\)4&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en)

31. www.oecd.org/chemicalsafety/risk-assessment/validationofqsarmodels.htm

32. www.seurat-1.eu/

33. Meeting JCR/OECD/EEB/PAN Europe, February 2016

3.2 Insufficient mechanistic information - AOPs an immature model for risk assessment

The 2013 OECD guidance³⁴ on AOP starts by acknowledging that

“to date, our limited knowledge about biological systems has hindered efforts to use mechanistic information as a basis for effects extrapolation”.

Indeed, even in the clearest toxicity cases the precise mechanisms by which for example smoking causes lung cancer in primates³⁵, or DDT causes egg-shell thinning in birds³⁶, or TBT causes imposex in marine and freshwater snails are still a matter of debate³⁷. It can take decades for causal mechanisms to be fully elucidated.

The OECD guidance further acknowledges that AOP is not ready for wide use in the foreseeable future:

“While the ultimate goal is to use AOPs in risk assessment, with the exception of a few specific cases, the level of information currently available is not sufficient to allow for risk assessment”.

Also German Health institute BfR in a review concludes³⁸:

“Yet, none of the currently discussed approaches for alternative testing can be deemed mature enough as to allow a complete replacement of the established testing systems. Major obstacles that remain are, among others, an incomplete understanding of molecular adversity and issues surrounding the validation of systems for high-throughput screening”.

34. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, 2013

35. www.theatlantic.com/magazine/archive/1956/01/lung-cancer-and-smoking-what-we-really-know/304760/

36. C. E. Lundholm (1997), DDE-Induced Eggshell Thinning in Birds: Effects of p,p'-DDE on the Calcium and Prostaglandin Metabolism of the Eggshell Gland, *Comp. Biol. Physiol.* Vol. 118C, No. 2, pp. 113-128

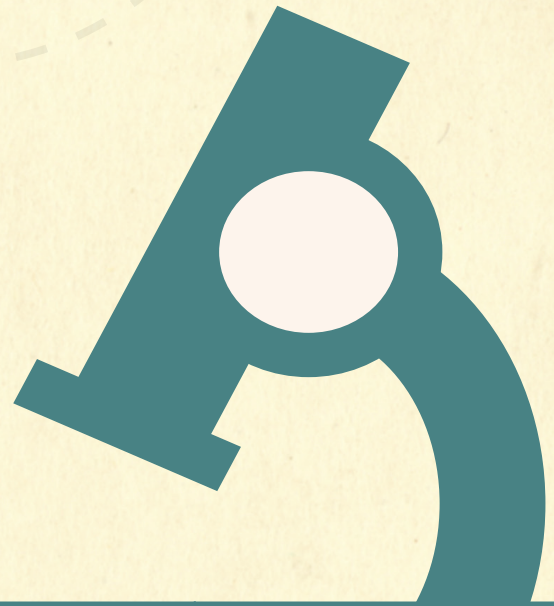
37. Cruz A, Rodrigues R, Pinheiro M, Mendo S, (2015). Transcriptomes analysis of *Aeromonas molluscorum* Av27 cells exposed to tributyltin (TBT): Unravelling the effects from the molecular level to the organism. *Marine Environ Res*, 109: 132-139

38. Tralau T., Oelgeschläger M., Gürtler R., Heinemeyer G., et al. (2015). Regulatory toxicology in the twenty-first century: challenges, perspectives and possible solutions. *Arch Toxicol* , 89: 823-850

Another example of the difficulties defining AOP is the report commissioned by Food Authority EFSA to elucidate the AOP for Parkinson Disease (PD)³⁹. In a systematic review 7348 published studies were considered and several possible “key events” (KE) identified, but the conclusion remains that

Therefore, the use of AOP in current EU risk assessment of chemicals could lead to erroneous conclusions.

“the challenging task in developing AOP of mechanisms leading to PD lies in identifying the sequence of molecular initiating events and intermediate events (especially for idiopathic PD) that eventually lead to the adverse outcome event. Many animal studies involve the knockout or mutational studies of the PD-related genes, which provide some basis of understanding on the mechanisms of familial PD pathogenesis. However, mechanisms leading to idiopathic PD remain unclear”.



More than a hundred leading cancer scientists warn against “mode of action” approaches

A review published by 134 leading cancer scientists⁴⁰ conclude that, “Our current understanding of the biology of cancer suggests that the cumulative effects of (non-carcinogenic) chemicals acting on different pathways that are relevant to cancer, and on a variety of cancer-relevant systems, organs, tissues and cells could conspire to produce carcinogenic synergies that will be overlooked using current risk assessment methods. Cumulative risk assessment methods that are based on ‘common mechanisms of toxicity’ or common ‘modes of action’ may therefore be underestimating cancer-related risks. In-utero and early life exposures, transgenerational effects and the interplay between the low-dose mechanistic effects of chemical mixtures in the environment and the vulnerabilities of subpopulations who are predisposed to cancer (i.e. via genetics or other influences) must also be considered. Current policies and practices do not adequately address these issues and should therefore be revisited if regulatory agencies hope to better understand and assess these risks”.

These observations seriously question the AOP-based approach.

39. Judy Choi, Alexandra Polcher, Anke Joas; Systematic literature review on Parkinson’s disease and Childhood Leukaemia and mode of actions for pesticides. Supporting Publications 2016:EN-955. 256 pp. Available online: www.efsa.europa.eu/publications

40. William H. Goodson III et al. Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: the challenge ahead, *Carcinogenesis*, 2015, Vol. 36, Supplement 1, S254-S296

3.3 Scientific basis for use of AOP to predict adverse effects is far from complete

Fundamental questions need to be answered first. Questions like what is the basis for the assumption that AOPs can play a solid role in predicting adverse effects? The OECD-guidance on AOPs⁴¹ refers to a much-cited US-EPA publication on AOP⁴², saying that the authors “believe AOPs provide a useful structure within which existing knowledge can be organized, from which key uncertainties and research priorities can be identified, and through which we can improve predictive approaches needed to advance regulatory ecotoxicology”. One can certainly hope it is more than a “belief”.

But where is the evidence that supports this hypothesis? Where is the scientific basis that proves that AOP is really capable of predicting adverse effects?

The US-EPA report just mentions a few cases to suggest robustness of AOP. This may be considered a poor approach. One will always be able to design a few cases on existing knowledge (and existing lack of knowledge) but this doesn't prove the relevance and robustness of this method for unknown chemicals.

In continuation, the OECD-guidance⁴³ refers to studies with many industry-linked experts such as the articles from professor Boobis^{44,45} that raise uncertainty about their independence and scientific objectivity. In particular, these studies are focussed mainly on the topic “human relevance”, a concept used repeatedly by industry to disqualify adverse effects from animal studies instead of identifying

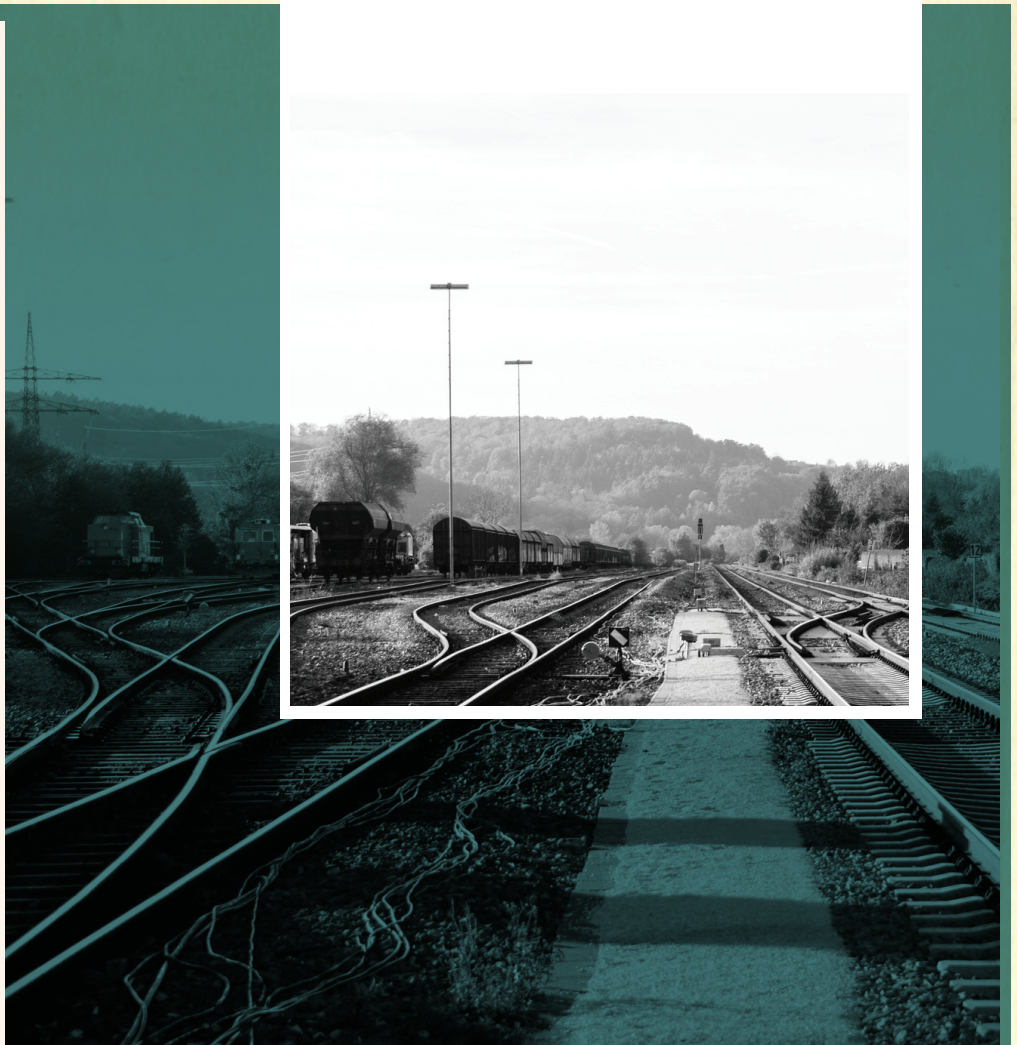


41. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, 2013
42. GERALD T. ANKLEY, RICHARD S. BENNETT, RUSSELL J. ERICKSON, DALE J. HOFF, MICHAEL W. HORNUNG, RODNEY D. JOHNSON, DAVID R. MOUNT, JOHN W. NICHOLS, CHRISTINE L. RUSSOM, PATRICIA K. SCHMIEDER, JOSE A. SERRRANO, JOSEPH E. TIETGE, and DANIEL L. VILLENEUVE, ADVERSE OUTCOME PATHWAYS: A CONCEPTUAL FRAMEWORK TO SUPPORT ECOTOXICOLOGY RESEARCH AND RISK ASSESSMENT, Environmental Toxicology and Chemistry, Vol. 29, No. 3, pp. 730-741, 2010
43. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, 2013
44. Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D., Farland, W. (2006). IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans. Crit. Rev. Toxicol. 36(10): 781-792.
45. Boobis, A.R., Doe, J.E., Heinrich-Hirsch, B., Meek, M.E., Munn, S., Ruchirawat, M., Schlatter, J., Seed, J., Vickers, C. (2008). IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans. Crit. Rev. Toxicol. 38(2):87-96.

adverse effects: *“Understanding of the mode of action of a chemical carcinogen will contribute to the consideration of the human relevance of the animal findings”*. This means overruling an adverse outcome from an animal study by using mechanistic information to prove it’s not relevant for humans. This should not be the objective of AOP. The objective should be to fill data gaps, not overrule adverse animal testing results.

Thus, the question that remains unanswered is how far AOP-information is capable of predicting an adverse outcome? What is the level of predictability, 99%, 95%, 80%, 50%? For now it is just a hypothesis, not supported by much evidence. And the concern is that the “train will keep on running”, forgetting about these fundamental questions.

The OECD moves consideration of the reliability/predictability to the end-users, the regulators. Regulators have to take reliability and predictability into account. Since these two elements are not taken into account by OECD and verification tests are not conducted, it is hard to understand how regulators would be able to do this, if they were interested doing so at all.





3.4. AOP, trapped in linear thinking?

It is relevant to look at mechanistic information for the adverse effects of chemicals. The better one understands the adverse effects of chemicals, the more precautionary action can be taken to prevent toxicity and disease. However, there is a concern that the concept of Adverse Outcome Pathways is too simplistic and it seems unlikely that one linear or branched 'AOP-pathway' can be drawn and proven, at an organism level. The suggestion of a chemical inducing a response in cell/body, leading through a few steps or key events to an adverse effect is unlikely. Many modes of actions do not follow a single or at its best a branched pathway, but a cascade of events involving triggering of the endocrine/nerve/immune system, receptor-mediated transport, transcription, synthesis, metabolism, transport of hormones across different tissues, feedback and homeostatic mechanisms. AOP might miss many important effects such as the hormonal effects during the early development vulnerable periods of life such (e.g. on foetus) and feedback mechanisms between organs and glands.

Especially the foetus should be of major concern in any risk assessment but still the attention (and current available data) is focussed on the healthy adult. This is a major flaw in current risk assessment⁴⁶. It is noted that a few AOPs are drafted on effects on the foetus, but still most attention is focussed on the 'healthy adult'.

46. Health Council of the Netherlands. Risks of prenatal exposure to substances. The Hague: Health Council of the Netherlands, 2014; publication no. 2014/05

The focus of AOP on single exposure of chemicals and not paying much attention to cumulative exposure is problematic and belongs to “old fashion” toxicology, as it’s not realistic since people are exposed to hundreds of chemicals every day. Although a consortium (with commercial trade group Freshfel and several experts linked to industry lobby group ILSI⁴⁷) -again granted with millions of taxpayers money- is looking at developing AOP for combined exposures and chemical mixtures, Euromix⁴⁸, this is not the central objective of the AOP framework and cumulative or synergistic effects are not mentioned at all in the OECD Guidance Document. The network involved in Euromix has many similarities to past consortiums (Acropolis⁴⁹) and working groups of Food Authority EFSA that tried to undermine cumulative risk assessment⁵⁰, with the intention to qualify the topic of “mixtures” as irrelevant.



The OECD speculates⁵¹ that as knowledge on AOPs expands, individual AOPs will naturally form a network, with some AOPs sharing KEs and adverse outcomes. AOP networks potentially can provide more realistic representation of AOPs than one single AOP. This knowledge might enable AOPs to contribute to the assessment of mixtures, as several AOPs could be considered together to capture the mode of action of the various substances in a mixture. One needs to have prior information on a chemical with unknown toxicity to link it with a certain AOP. For most chemicals however, there is little information available in regards to cumulative,

synergistic or antagonistic effects when considering combined exposures. OECD expects that the more we know about the AOPs that chemicals induce, the more opportunities there are to understand the potential for cumulative, synergistic or antagonistic effects between chemicals. AOP currently on the wiki platform are qualitative descriptions, which might not enable any evaluation of cumulative or synergistic effects. OECD further hopes that quantitative AOPs might provide an estimate of what magnitude and/or duration of change in the upstream KE is needed to evoke some magnitude of change in the downstream KE. There may be potential in the future for quantitative AOPs, coupled with AOP networks, to inform cumulative or synergistic issues. All in all, AOP does not offer much on cumulative exposure by now and use of current AOPs will mean that cumulative exposures are disregarded.

47. www.euromixproject.eu/news/press-releases/euromix-project-kick-off/agenda-kick-off-meeting/

48. www.euromixproject.eu

49. www.acropolis-eu.com/

50. PAN Europe report A Poisonous Injection 2014. www.pan-europe.info/resources/reports

51. Meeting JCR/OECD/EEB/PAN Europe, February 2016

It is well known, that chemicals with a different mode of action might contribute to adversity of the same target in the body⁵². Now that we are finally getting rid of the historical mistake to solely look at the toxic effects of a single chemical⁵³ and we are moving to cumulative exposure, AOP should therefore prioritise the cumulative exposure scenarios and take them into account in all AOPs. Otherwise we are returning to decades-ago times, ignoring scientific consensus (and EU laws) on cumulative effects of chemicals.

Borgert et al.⁵⁴ are not optimistic about the use of AOP because of its limitations to account for mixture toxicity:

“Predictions based on mechanistic similarity may simply be impractical for most chemicals due to uncertainties in the mechanisms or modes of action by which they operate. Obtaining the required mechanistic information may be technically impossible for chemicals that produce effects by multiple mechanisms. Until a scientifically defensible, generally applicable theory for mixtures is formulated and a sufficiently broad base of data directed toward examining this theory is generated, regulatory approaches that utilize mode of action to predict mixture toxicity will remain tenuous”.

52. www.efsa.europa.eu/en/efsajournal/pub/3472

53. Regulation 396/2005 on pesticide residues; Regulation 1107/2009 on the approval of pesticides

54. Christopher J. Borgert, Terry F. Quill, Lynn S. McCarty, Ann M. Mason, Can mode of action predict mixture toxicity for risk assessment?, Toxicology and Applied Pharmacology 201 (2004) 85– 96

The same goes on for Goodson et al.⁵⁵. They particularly criticize AOP and MoA, and conclude:

"..current regulations in many countries (that consider only the cumulative effects of exposures to individual carcinogens that act via a common sequence of key events and processes on a common target/tissue to produce cancer) should be revisited. Our current understanding of the biology of cancer suggests that the cumulative effects of (non-carcinogenic) chemicals acting on different pathways that are relevant to cancer, and on a variety of cancer-relevant systems, organs, tissues and cells could conspire to produce carcinogenic synergies that will be overlooked using current risk assessment methods.

Cumulative risk assessment methods that are based on 'common mechanisms of toxicity' or common 'modes of action' may therefore be underestimating cancer-related risks. In-utero and early life exposures, transgenerational effects and the interplay between the low-dose mechanistic effects of chemical mixtures in the environment and the vulnerabilities of subpopulations who are predisposed to cancer (i.e. via genetics or other influences) must also be considered. Current policies and practices do not adequately address these issues and should therefore be revisited if regulatory agencies hope to better understand and assess these risks".

55. Goodson WH et al. (>100 authors) (2015), Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: the challenge ahead, *Carcinogenesis*, Vol. 36, Supplement 1, S254-S296

3.5 Link between mode of action and adverse effect: clear or not clear?

Lorenzetti et al.⁵⁶ describe the different views on screening tests for endocrine disruption. Some authors insist that “*endocrine disruption is just a mode-of-action that may or may not result in adverse effects*” and that endocrine disruptors have to be handled like other non-genotoxic agents (supported by authors like Dietrich and DeKant⁵⁷ that protested against the EU policy to regulate endocrine disruptors). According to this viewpoint, endocrine disruption is somewhat like “*much ado about nothing*” because the endocrine effects might fall within the maintenance of the physiological homeostasis or in most cases, effects that matter are those identified by the conventional apical endpoints of *in vivo* assays. The straight application of this viewpoint might be pushed quite far away: a reduction of spermatogenesis without a demonstrated reduced fertility in laboratory animals or an altered brain biochemistry without proven neurobehavioural disturbances could be questioned with regards to their “actual” adversity⁵⁸. Such and similar effects may be compensated when exposure takes place during adulthood, but this concept totally neglects decades of research on the effects of chemicals when exposure at very low doses takes place during the early life when an organism is still under development.

An opposite position retains that pointing out an endocrine-like mode of action (MoA) indicates *per se* a potential hazard because of the critical importance of altered endocrine homeostasis during vulnerable life stages (i.e. pregnancy, foetal development, puberty). Hence, small changes in hormone signalling can be compensated in the adult organism, whereas changes of the same or even lower magnitude may lead to adverse consequences when they occur during the susceptible developmental windows of early life.



56. Stefano Lorenzetti, Daniele Marcocchia and Alberto Mantovani, Biomarkers of effect in endocrine disruption: how to link a functional assay to an adverse outcome pathway, *Ann Ist Super Sanità* 2015 | Vol. 51, No. 2: 167-171

57. Letter Dekant, Greim and other to prof. Glover, <http://dx.doi.org/10.1016/j.tiv.2013.07.001>

58. Stefano Lorenzetti, Daniele Marcocchia and Alberto Mantovani, Biomarkers of effect in endocrine disruption: how to link a functional assay to an adverse outcome pathway, *Ann Ist Super Sanità* 2015 | Vol. 51, No. 2: 167-171

This failure of linking a key event to an adverse outcome and *vice versa* was also observed by Krystle et al.⁵⁹ following exposure of zebrafish embryo to the organophosphate paraoxon:

1

"In summary, our data suggest that

normal AChE activity is not required for secondary motoneuron development and

2

AChE inhibition may not be associated with an increased frequency of spontaneous tail contractions at 26 hpf following paraoxon exposure. Although paraoxon was a potent AChE inhibitor within zebrafish embryos, this initiating event was not linked to adverse outcomes on secondary motoneuron development at 96 hpf and was fully reversible within 48 h following transfer of embryos to clean water. Moreover, the most sensitive paraoxon-induced adverse outcome in this study – an increased frequency of spontaneous tail contractions at 26 hpf – occurred in the absence of significant AChE activity".

Thus, organophosphates like paraoxon and chlorpyrifos-oxon that share a common target (i.e. AChE activity) do not share identical mechanisms of toxicity. This study is a clear case that questions the reductionist's approach taken in AOP.

59. Krystle L. Yozzo, Sean P. McGee, David C. Volz, Adverse outcome pathways during zebrafish embryogenesis: A case study with paraoxon, *Aquatic Toxicology* 126 (2013) 346–354

3.6 AOPs still focusing on operators and not the general population

In the past, the focus of toxicity testing was mainly to protect the operator who is handling the toxic substance, being the one with the highest risk of exposure. The chemical industry has now clear rules to minimize worker's exposure using protecting clothing, and minimising leakage of toxic chemicals in the working environment. But the general public is also exposed to toxic chemicals that are released into the environment due to industrial and agricultural activities or through consumer's goods. Only recently the industry has been directed to do a couple of whole life-time experiments to mimic the real-life situation of the general public: chronic exposures where parents, babies in the womb, babies and children are exposed to the chemical. These tests are far from complete as they don't include all possible adverse effects that chemicals may cause (e.g. endocrine, immune, neurologic) and do not test low, real-life environmental concentrations of chemicals or all possible exposure scenarios (one off exposure or of short period during early life development with adverse effects manifesting later in life, e.g. neurodegenerative diseases like Parkinson and Alzheimer).

Next to missing cumulative and synergistic exposure to chemicals, only very few AOPs highlight the importance to focus on the specific vulnerability of the unborn. Thus, the AOP framework may get trapped in the decades-old risk assessment thinking. Hundreds of studies of independent scientists in academic laboratories show how a broad selection of chemicals can interfere with the normal development of offspring at extremely low levels of exposure (Bisphenol A⁶⁰, Atrazin⁶¹, Fenarimol⁶² etc.). These studies were done with the knowledge that the embryo and foetus develop under the control of hormones at parts per billion and parts per trillion levels, and that as the baby matures hormone concentrations are regulated by sensitive, thermostat-like feedback control systems in the brain.

These (low-dose) studies are not included in databases for regulatory purposes since they are dominated by OECD/GLP-tests with (extreme) high exposure doses. With a lack of data, it will be difficult to develop an AOP.

Many chemicals with endocrine disrupting properties act at (very) low doses⁶³.

60. LOEL mice 2 ppb: Vom Saal, F.S.; Cooke, P.S.; Buchanan, D.L.; Palanza, P.; Thayer, K.A.; Nagel, S.C.; Parmigiani, S.; Welshons, W.V. 1998. A physiologically based approach to the study of Bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicology and Industrial Health* 14, 1/2: 239-260
61. LOEL mice 1 ppb: V. Belloni, E. Alleva, F. Dessi-Fulgheri, M. Zaccaroni and D. Santucci, Effects of low doses of atrazine on the neurobehavioural development of mice, *Ethology Ecology & Evolution* 19: 309-322, 2007
62. LOEL mice 2 ppb: Mira Park, Jiyoun Han, Jeong-Jae Ko, Woo-Sik Lee, Tae Ki Yoon, Kangseok Lee, Jeehyeon Bae, Maternal exposure to fenarimol promotes reproductive performance in mouse offspring, *Toxicology Letters* 205 (2011) 241- 249
63. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP, Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses, *Endocrine Reviews*, June 2012, 33(3):378-455

The traditional idea of relying on the threshold linear dose-response curve does not work for many chemicals with endocrine disrupting properties. In several cases such chemicals have been proven to act according to a non-monotonic, inverted U-shaped dose-response curve⁶⁴, which means that lower doses may trigger a biological effect that is not apparent at higher doses, due to saturation of the specific molecular responses.

Some examples of such a non-linear dose-response curve are: low doses of the anti-miscarriage drug DES cause prostate enlargement while high doses cause the opposite⁶⁵. Rat experiments on DEHP, a phthalate found in plastics, show that low doses suppress an enzyme needed for proper development of the male brain, while high doses stimulate the enzyme⁶⁶. Bisphenol A, a chemical compound used in plastics, induces the development of mammary tumours in female mice and pulmonary metastasis following chronic exposure only to low doses, as at higher doses these effects do not occur⁶⁷. The well-known drug tamoxifen, given to treat certain breast cancers, is known to have opposite effects at different levels in the body⁶⁸.

64. John Peterson Myers, R. Thomas Zoeller, and Frederick S. vom Saal, A Clash of Old and New Scientific Concepts in Toxicity, with Important Implications for Public Health, volume 117 | number 11 | November 2009 • Environmental Health Perspectives
65. Vom Saal FS, Timms BG, et al. (1997), Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses, Proc. Natl. Acad. Sci. USA Vol. 94, pp. 2056–2061, March 1997
66. Andrade AJ, Grande SW, Talsness CE, Grote K, Chahoud I 2006 A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl)-phthalate (DEHP): non-monotonic dose-response and low dose effects on rat brain aromatase activity. Toxicology 227:185–192
67. Jenkins S, Wang J, Eltoum I, Desmond R, Lamartiniere CA, 2011. Chronic Oral Exposure to Bisphenol A Results in a Nonmonotonic Dose Response in Mammary Carcinogenesis and Metastasis in MMTV-erbB2 Mice. Env Health Persp 199:1604-1609
68. XinTian Zhang, Ling Ding, LianGuo Kang, Zhao-Yi Wang, Estrogen Receptor-Alpha 36 Mediates Mitogenic Antiestrogen Signaling in ER-Negative Breast Cancer Cells, PLoS ONE | www.plosone.org 1 January 2012 | Volume 7 | Issue 1

3.7 Data delivered to AOP, are they robust?

Very worrying in the text in the OECD-guidance⁶⁹ are the lines on “partial” and “qualitative” AOPs:

“A partial AOP (i.e. one where not all key events are known), such as may come from a scoping exercise, may be useful in priority setting for further testing and development. Similarly, partial AOPs may be used in hazard identification, as is currently performed with the OECD QSAR Toolbox. At this time, physiologically-based pharmacokinetic (PBPK) modelling and toxicokinetics information on absorption, distribution, metabolism, and excretion (ADME) are out of the context of the AOP but will have to be addressed to develop a quantitative AOP required for a complete risk assessment”.

OECD should make sure that these partial/qualitative AOPs are used for specific purposes and not open the way to the use of all kinds of information, QSAR, “read-across”, PBPK-modelling and TTC (Threshold of Toxicological Concern), alone or in combination, in the application in risk assessment to use a “partial/qualitative” AOP that might be solely based on beliefs, assumptions and speculations, and lack robust mechanistic information.



“The concept of quantitative structure–activity relationships (QSAR) is inherently associated with optimism, a mindset ever hopeful for predictive correlations and the prospects of novel insight or hypothesis. However, lately the concept engenders quite the opposite reaction from the scientific community-at-large, a negative view which is not entirely without merit”.

QSAR has been around for a long time but the level of predictability is still one of the main questions around its use. Doweiko writes in 2008⁷⁰:

69. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, 2013

70. Arthur M. Doweiko, QSAR: dead or alive?, J Comput Aided Mol Des (2008) 22:81–89

OECD has been working on QSAR for many years and in 2004 its principles⁷¹ were approved during a workshop organised by industry (CEFIC, ICCA). Here again it looks like QSAR is dominated much by regulatory experts, many of them with links to industry, with little interest and input from academic scientists.

EU Joint Research Center (JRC) seems cautious on QSAR:

“the availability of (Q)SAR models for chronic toxicity endpoints is currently very limited. Since a large number of potential targets and mechanisms are associated with repeated dose effects, it is unlikely that any single model or software tool will be capable of making reliable predictions for all chemicals of interest to dietary risk assessment. In view of the limited availability of QSARs and predictive software for chronic toxicity effects, the read-across approach merits further investigation, and automated software should be developed further”⁷².

This is subsequently pursued in the industry/JRC research program SEURAT.

If read-across is used, Cronin⁷³ argues that a robust ‘read-across’ should be used, based on many conditions, a range of data matrices, many data on chemical and biological properties, defining similarities on many levels and the uncertainty. Regarding the last topic Cronin states:

“Currently, determining how much uncertainty is acceptable for a read-across prediction is still largely subjective. It is defined on a case-by-case basis and influenced heavily by the purpose of the prediction, the endpoint assessed, and whether the read-across predicts the presence or absence of toxicity”.

Conclusion: it is largely subjective!

QSAR and “read-across” therefore are not more than prediction tools with much uncertainty and subjectivity and cannot replace testing-based risk assessment. These tools can be used as providing some information and guidance to the producers about which chemicals to reject due to their possible toxicity and which ones to select to carry out further testing and assessment.

71. www.oecd.org/chemicalsafety/risk-assessment/historyoftheoecdqsarproject.htm 1) a defined endpoint, 2) an unambiguous algorithm, 3) a defined domain of applicability, 4) appropriate measures of goodness-of-fit, robustness and predictivity, 5) a mechanistic interpretation, if possible

72. Silvia Lapenna, Mojca Fuart-Gatnik and Andrew Worth, Review of QSAR Models and Software Tools for predicting Acute and Chronic Systemic Toxicity, EU JRC, 2010.

73. T.W. Schultz, P. Amcoff, E. Berggren, F. Gautier, M. Klaric, D.J. Knight, C. Mahony, M. Schwarz, A. White, M.T.D. Cronin, A strategy for structuring and reporting a read-across prediction of toxicity, Regulatory Toxicology and Pharmacology 72 (2015) 586–601

Other tools, like physiologically-based pharmacokinetic (PBPK) modelling, are even more in their infancy, but are already criticized for underestimating risks⁷⁴. Also data are lacking. Moretto and co-workers⁷⁵ would very much like to use PBPK for *in vitro* to *in vivo* extrapolation but they state: *“Regarding the extrapolation to the in vivo situation, PBPK modelling proves to be a powerful tool; however, it cannot be widely applied, at least in the short term, because a lot of compound specific data on ADME (absorption, distribution, metabolism, excretion) are needed”*.

A final tool to highlight is TTC, the Threshold of Toxicological Concern. The tool is developed and very much promoted by industry lobby group ILSI (International Life Sciences Institute) and finally accepted in a controversial “conflicts-of-interests” case⁷⁶ by Food Authority EFSA. TTC can be questioned for many of its elements, a decades-old non-retrievable database, arbitrarily putting chemicals in and outgroups, probabilistic modelling, excluding 5-percentile of the data, etc.⁷⁷. The tool was approved for ‘priority setting’ and “screening’ for data gaps, but soon this was forgotten and TTC became being used as a “safe” level in regulatory decision-taking⁷⁸. All these systems of prediction are only as strong as their input is (GIGO-principle).



74. www.independentsciencenews.org/health/why-the-united-states-leaves-deadly-chemicals-on-the-market/

75. Angelo Moretto, Francesca Di Renzo, Erminio Giavini, Francesca Metruccio, Elena Menegola, The use of *in vitro* testing to refine cumulative assessment groups of pesticides: The example of teratogenic conazoles, *Food and Chemical Toxicology* 79 (2015) 65–69

76. PAN E report on TTC

77. www.efsa.europa.eu/en/events/event/141202

78. Use of TTC in full risk assessment of metabolites of pesticides promoted by EFSA, www.efsa.europa.eu/en/consultations/call/160307

3.8 Industry's lobby agenda in the EU-program SEURAT

The industry/ILSI program COSMOS⁷⁹ was part of the SEURAT program and contained many of industry's previous lobby items that raise concerns for public health:



It remains to be seen if these elements will be robust parts of AOP or just 'qualitative' ones based on assumptions and speculations and 'expert judgement', the entire 'belief spectrum' of the one doing the assessment.

79. www.cosmostox.eu/home/welcome/

3.9 Other alternatives to mammalian testing

Krystle et al.⁸⁰ discuss alternatives for animal testing and conclude that “*exposure of fish embryos is one of the promising tools. To date, the most promising alternative and cost-efficient vertebrate models for rapid screening of chemicals for developmental toxicity are early life-stages of teleosts (bony fishes). As fish embryos (pre-hatch stages) and eleutheroembryos – the time period between hatch and independent feeding – are non-protected life-stages, these early life-stages are considered alternative testing models within the European Union and United States. In contrast to cell-based assays, fish embryos provide the complexity and interaction of an intact organism, enabling the evaluation of adverse chemical effects on multiple target organs and developmental stages during embryogenesis*”.

Fish naturally produce hundreds of eggs that are mostly eaten by other fish. However, in the laboratory, fish eggs can be separated from the adults to protect them and allow egg hatching. According to the EU Directive 2010/63/EU on the protection of animals used for scientific purposes, fish embryos are not subject to animal regulation until external feeding commences, i.e. around 5 days post fertilization. Zebrafish models have been used as a developmental and embryological model since the 1930s but in the last decades they have been used successfully to understand the genetic mechanisms underlying human diseases⁸¹. As zebrafish can be used for modelling human diseases and drug discovery, they can also be used successfully to test specific chemicals for human-relevant adverse effects and diseases.



80. Krystle L. Yozzo, Sean P. McGee, David C. Volz, Adverse outcome pathways during zebrafish embryogenesis: A case study with paraoxon, *Aquatic Toxicology* 126 (2013) 346– 354

81. Lieschke GJ, Currie PD, 2007. Animal models of human disease: zebrafish swim into view. *Nature Reviews Genetics* 8:353-367



Invertebrate models can also provide human-relevant mechanistic information, on the interaction of chemicals with enzymes and metabolism that may lead to adverse effects. For example *Drosophila* and *Caenorhabditis elegans*, possess pathways important for human disease and development, and could be used for example to predict metabolic, developmental and immune system diseases⁸².

Such tests can be applied as a first screening to decide against the use of potential harmful chemicals.

It is not certain if these types of experiments are allowed for cosmetics safety testing but for REACH chemicals they surely should be promoted.

82. Schlegel A, Stainier DYR (2007) Lessons from "Lower" Organisms: What Worms, Flies, and Zebrafish Can Teach Us about Human Energy Metabolism. PLoS Genet 3(11): e199.



3.10 AOP already used by Commission

While being far from ready for regulatory purposes, AOP and similar tools (TTC and “human relevance”) have been introduced already in EU regulatory decision-making processes.

One example of the use of AOP is the proposal of DG SANTE, the EU institution responsible for pesticide testing, in a guideline to allow industry to use AOP to overrule OECD-testing results⁸³. This is of course very controversial, substituting facts by prediction, and exactly the type of misuse that is unfortunately “out of scope” for OECD. A proper political discussion has not taken place yet on the use of AOP, neither the parliament nor the public are consulted. In fact the inclusion of AOP in risk assessment of pesticides is done secretly, behind the closed doors of the SANTE Standing Committee on pesticides; the Committee of 28 EU member states deciding on the proposals put forward by Commission service DG SANTE.

In the opinions on the safety of pesticides, many times “expert judgement” is applied⁸⁴. This “expert judgement” is a very vague, intransparent and obscure procedure where words such as “assuming” and “believing” are commonly being used. Despite being based mainly on the beliefs-spectrum of the one who is doing the risk assessment, this practice is unfortunately very widespread in European regulatory affairs.

83. Personal communication K.Nienstad, DG SANTE unit E3, 2015.

84. PAN Europe report 2014. A Poisonous injection www.pan-europe.info/resources/reports



A further example of predictive tools overruling test outcomes is the case of QSAR data overruling existing test guidelines:

Sweden criticised EU Commission service DG SANTE on a proposal to disqualify observed mutations for the pesticide Etridiazole by QSAR information⁸⁵:

“As part of the confirmatory data on etridiazole the relevance of the plant metabolite 5-hydroxy-ethoxyetridiazole acid was assessed. A mouse lymphoma assay was performed according to current guideline. The results show a clear positive response, being almost perfectly dose dependent, in large colonies, indicating mutations associated with point mutations. For the small colonies a positive response can be seen over the three highest doses, indicating mutations associated with chromosomal aberrations.

Regarding the Structure Activity Relationship analysis, the intention with such analysis is to fill data gaps, not to overrule experimental data. In cases such as this, with positive experimental results, these results should be given precedence over a negative computer analysis. In our opinion the plant metabolite 5-hydroxy-ethoxyetridiazole acid should currently be regarded as toxicologically relevant”.

Again a case of facts is being overruled by the outcome of prediction tools.

The tendency to include AOP before it is ready and without proper discussions is worrying.

85. Document obtained after an access-to-documents request to DG SANTE, 2015

4

Use and misuse of AOP, the Adverse Outcome Pathway

OECD AND JRC seem to be a bit “naïve” on the misuse of AOPs in regulatory assessment of chemicals. While they assume that it is about using the tool in case of data gaps, it could be quite different at implementation level with a lot of ‘politics’ when Commission and national ministries are involved, fuelled by heavy industry (and farmer) lobbying.

4.1 AOP, a “déjà vu” of industry advocacy?

Those with decades of experiences with chemical risk assessment know that tools can be used and misused. These tools are agreed upon in “nice” meetings where everybody behaves like scientists, but unfortunately no attention is given to the implementation phase of these tools where hard advocacy and politics rule and those with most resources and lobbyists tend to get their way. Anything goes at that phase. Misuse should be prevented actively at that early phase, but there is no sign that something is done in AOP case.

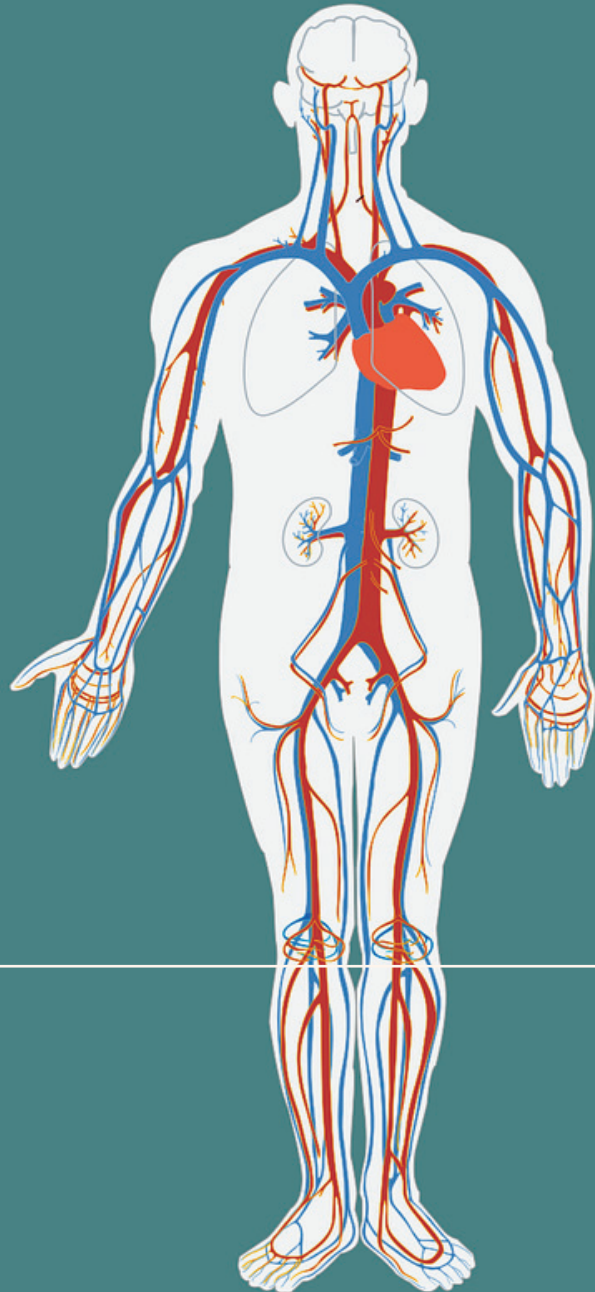
This potential misuse might not be obvious to those involved in the developmental phase and may only become apparent when the AOP-tool will be used in practice at the time chemical companies fight for the approval of their substances and use any opportunity to claim safety. Now they will get the opportunity to claim the use of AOP. Government officials should be more aware about the potential misuses and block them already in the construction phase. AOP has many similarities with the old discussion on MoA (Mechanism of action), promoted by industry as a central element of risk assessment⁸⁶ and the concerns can be illustrated by the (mis)use of tools based on MoA. Two examples are presented below.

86. Neil Carmichael, Melanie Bausen, Alan R. Boobis, Samuel M. Cohen, Michelle Embry, Claudia Fruijtner-Pölloth, Helmut Greim, Richard Lewis, M.E. (Bette) Meek, Howard Mellor, Carolyn Vickers, and John Doe, Using mode of action information to improve regulatory decision-making: An ECETOC/ILSI RF/HESI workshop overview, *Critical Reviews in Toxicology*, 2011; 41(3): 175–186

4.2 Example: the tool “human relevance” to disqualify adverse effects observed in animal studies

A first example of misuse of MoA is the tool “human relevance”, very much promoted by industry⁸⁷, and their views even made it to the WHO/ IPCS-report on ‘human relevance’ and published by experts connected to industry lobby groups ILSI⁸⁸ and ECETOC⁸⁹ (Boobis et al., 2008⁹⁰).

Many adverse effects seen in animals tested for the pesticide regulation –using the IPCS-framework⁹¹- are now considered non-relevant based on assumptions and speculations about a MoA differing between rodents and humans. These speculations and assumptions overrule the results of experimental animal testing, without further investigation, and this happens on a large scale⁹². Below we present several such cases for illustration purposes.



87. M. E. (Bette) Meek, John R. Bucher, Samuel M. Cohen, Vicki Dellarco, Richard N. Hill, Lois D. Lehman-McKeeman, David G. Longfellow, Timothy Pastoor, Jennifer Seed, and Dorothy E. Patton, A Framework for Human Relevance Analysis of Information on Carcinogenic Modes of Action, *Critical Reviews in Toxicology*, 33(6):591–653, 2003

88. ILSI, International Life Science Institute, an industry lobby group

89. ECETOC, European Center for Ecotoxicology and Toxicology of Chemicals, EU industry expert center.

90. Boobis, Doe, Meek, Schlatter et al. IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans, *Critical Reviews in Toxicology*, 38:87–96, 2008

91. Boobis, A.R., Doe, J.E., Heinrich-Hirsch, B., Meek, M.E., Munn, S., Ruchirawat, M., Schlatter, J., Seed, J., Vickers, C. (2008). IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans. *Crit. Rev. Toxicol.* 38(2):87–96., 2008.

92. www.pan-europe.info/press-releases/2015/01/new-non-commercial-research-proves-industry%E2%80%99s-claims-pesticide-bans-are-see-for-instance-the-regulatory-decisions-and-EFSA-opinions-on-Abamectin,Bupirimate,Epoxyconazole,Ioxynil,Linuron,MetribuzinandTebuconazole

4.2.1 Abamectin

Regarding the chemical Abamectin, high mortality in neonatal rats was observed; the risk assessment however states⁹³,



“In rats, expression of P-glycoprotein in the brain develops to adult levels during the first 20 days after birth, and the expression of P-glycoprotein in the jejunum does not start before postnatal day 8. Since this susceptible period with limited P-glycoprotein expression after birth is not present in man, effects observed in neonatal rats during lactation are considered less appropriate for human risk evaluation of abamectin and the 8,9-Z isomers”.

UK states:

*Development in the neonatal rat is considered to be sufficiently different to humans (with respect to P-glycoprotein expression) to make the findings in the multi-generation study **not relevant to the risk assessment**.*

Syngenta’s view is:

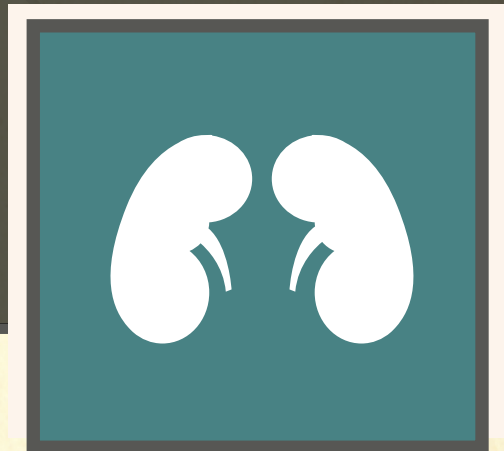
*that the use of the **polymorphic CF-1 mouse** is not relevant for human risk assessment on the basis of the unique polymorphism of the murine *mdr1a* gene and the available evidence concerning human polymorphisms of the MDR1 gene.*

THIS IS PURE SPECULATION ABOUT A SUSCEPTIBLE PERIOD IN MAN (NEONATAL?) NOT BEING PRESENT WHILE NO EVIDENCE IS DELIVERED.

93. www.efsa.europa.eu/en/efsajournal/pub/147r

4.2.2 Bupirimate

Regarding the pesticide Bupirimate, kidney cancers were observed, but risk assessment concludes⁹⁴,



BE:

Thyroid adenomas were reported in males at top dose: mechanistic study does not support a liver enzyme induction effect (see 28 day rat study, supplementary study) where TSH, T3 and T4 were not induced. Therefore, we consider that bupirimate should be considered as carcinogenic in rats.

NL:

Historical control data have been provided by industry for all tumour types that appeared to be increased in the rat study except for thyroid tumours. These showed that there was no significant increase in tumour incidence except for thyroid tumours, which are known not to be relevant for human risk assessment

EFSA:

independent of the classification issue (due to the occurrence of thyroid and skin tumours in rats), there is a clear threshold for these effects, not affecting the risk assessment.

Industry:

The increases in absolute and relative thyroid weight in dogs that received 3 and 15 mg/kg bw/day were within the historical control range and not statistically significant compared with control values.

EFSA, finally:

In the rat thyroid follicular adenomas occurred at higher doses, but they were considered of no relevance to humans”.

THIS IS WHAT CAN BE EXPECTED WHEN AOP WILL BE USED: MECHANISTIC DIFFERENCES ARE OBSERVED, AND -AS A DEFAULT- ADVERSE EFFECTS DISMISSED.

94. www.efsa.europa.eu/en/efsajournal/pub/1786

4.2.3 Epoxiconazole

Regarding the pesticide Epoxiconazole liver tumours were observed in mice; the risk assessment however concludes⁹⁵:



"In a 24-months study in rats liver toxicity was observed and additionally ovary- and adrenal gland tumours were seen.

In a 18-month carcinogenicity study in mice a treatment related increase in liver tumours has been observed. Based on mechanistic data (these data were not provided) the tumours in rats were considered as non-relevant for human risk assessment".

THE MECHANISTIC DATA THAT RULE OUT THE SIGNIFICANCE OF THE LIVER TUMOURS FOR HUMANS ARE NOT PROVIDED.

95 www.efsa.europa.eu/en/efsajournal/pub/138r

4.2.4 Ioxynil

Regarding the pesticide Ioxynil thyroid cancer was observed in rats; the risk assessment (DG SANTE review report) however concludes⁹⁶:



"Liver tumours in rat and male mice. Thyroid tumours (rat), uterus tumours (mice). Mechanistic studies suggest however that the mechanism of Ioxynil induced thyroid carcinogenesis in the rat is the result of a species specific perturbation of thyroid hormone homeostasis".

THIS IS PURE SPECULATION.

96. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance_detail&language=EN&selectedID=1480

4.2.5 Linuron

Regarding the pesticide Linuron changes in testosterone level were observed; however risk assessment (DAR 1996) concludes:



*" A plausible non-genotoxic mechanism for the tumorigenicity could be associated with the claimed anti-androgenic properties of linuron. Any compound which disrupts the regulation of the hypothalamus-pituitary-testicular (HPT) axis can result in sustained hypersecretion of luteinizing hormone (LH) which could be a mechanism for production of Leydig cell tumours. The same hormonal changes have been proposed to account for the uterine and ovarian tumours in female rats, via an interaction with normal age-related phenomena, whereby aged female rats enter a stage of persistent oestrus at approximately 12-15 months of age. Linuron is structurally related to compounds which have been shown to act via this mechanism. If it is accepted that linuron is producing tumours via an antiandrogenic mechanism, where sustained hypersecretion of LH is responsible for the alteration in tumour incidence, then exposure to linuron at levels which do not disrupt the HPT axis **should pose no risk for tumour development** because a definable threshold level should exist".*

**THIS IS A NON-EVIDENCE
BASED SPECULATION ABOUT
THRESHOLDS.**

4.2.6 Metribuzin

Regarding the pesticide Metribuzin effects on thyroid hormone levels were observed; however risk assessment⁹⁷ concludes:



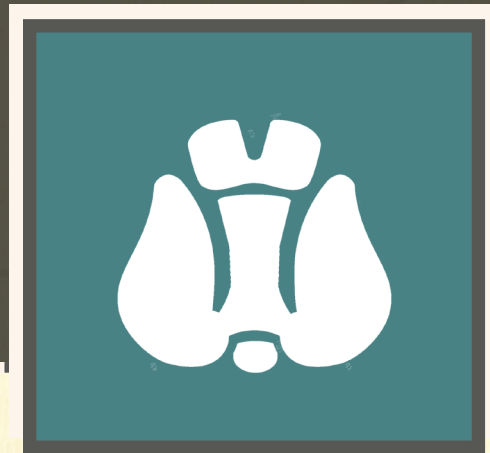
"The liver was found to be the main target organ in rats, mice and dogs. In rats effects on thyroid (histology, T3 and T4 changes) were recorded, as after short-term exposure.

The thyroid effects were interpreted in terms of a rodent-specific response due to liver enzyme induction. Neither functional impairment nor increased tumour incidence in the thyroid was noted".

97. www.efsa.europa.eu/en/efsajournal/pub/88r

4.2.7 Tebuconazole

Regarding the pesticide Tebuconazole both liver tumours and thyroid tumours were observed; however risk assessment⁹⁸ concludes:




"Liver tumours in sensitive mice strain. Not relevant for humans".

"C-cell carcinomas and adenomas of the thyroid were increased in all treated males (not dose-related, not statistically significant). The historical data and data from this study provide strong evidence that the incidence of thyroid tumours observed in the Tebuconazole study were not treatment related".

TUMOURS INCIDENTS ARE HIGHER THAN CONTROLS BUT STILL NOT RELEVANT TO HUMANS OR NOT RELATED TO TREATMENT. THIS IS A SPECULATION, NO EVIDENCE IS PROVIDED.

THE EFFECTS ON THE THYROID FOLLOWING EXPOSURE TO THE PESTICIDES BUPIRIMATE, IOXYNIL METRIBUZIN TEBUCONAZOLE ARE DISMISSED WITHOUT FURTHER TESTING TO CONFIRM A SUGGESTED AND ASSUMED NON-RELEVANCE OF THE OBSERVED THYROID EFFECTS. THERE IS NO SCIENCE BEHIND THESE ASSUMPTIONS BUT EVERY APPLICANT OF CHEMICALS WILL GO TO BIG LENGTHS TO CLAIM A NON-RELEVANCE.

98. www.efsa.europa.eu/en/efsajournal/pub/3485

A top-down photograph of a person's hands typing on a silver laptop keyboard. To the left of the laptop is a dark grey notebook with a pen resting on it. The background is a light-colored wooden desk. A semi-transparent teal box is overlaid on the bottom half of the image, containing white text.

Confronted by expensive industry consultancies and paid university professors, the few government experts probably are no match to the resources available to industry, with their continued and standard claims of non-relevance of observed effects, claims and calculations of effects being non-treatment related, claims and speculations about effects being species-specific, claims on QSAR and “read-across” overruling observed effects in animal studies, additional reasoning of many pages likely by paid academics and many times studies published in industry-friendly journals casting doubt on adverse effects observed.

4.3 Example: Industry-efforts to 'neutralise' the effects of introducing cumulative risk assessment of chemicals

A second example concerns the substantial efforts that industry, notably ILSI, has put in advocating a regulatory policy for mixture toxicity based on MoA⁹⁹. Because there will always be (tiny) differences in MoA, even between different chemicals of a same group (like in the group of Triazoles or Organophosphates), chemicals can – in case of a very strict use of MoA - be dismissed from the same cumulative group. This is what a panel from Food Authority EFSA, with several advocates of industry's views, proposed for a long time until Commission intervened. This "use" of MoA in the end tended to disqualify cumulative effects as non-existent:

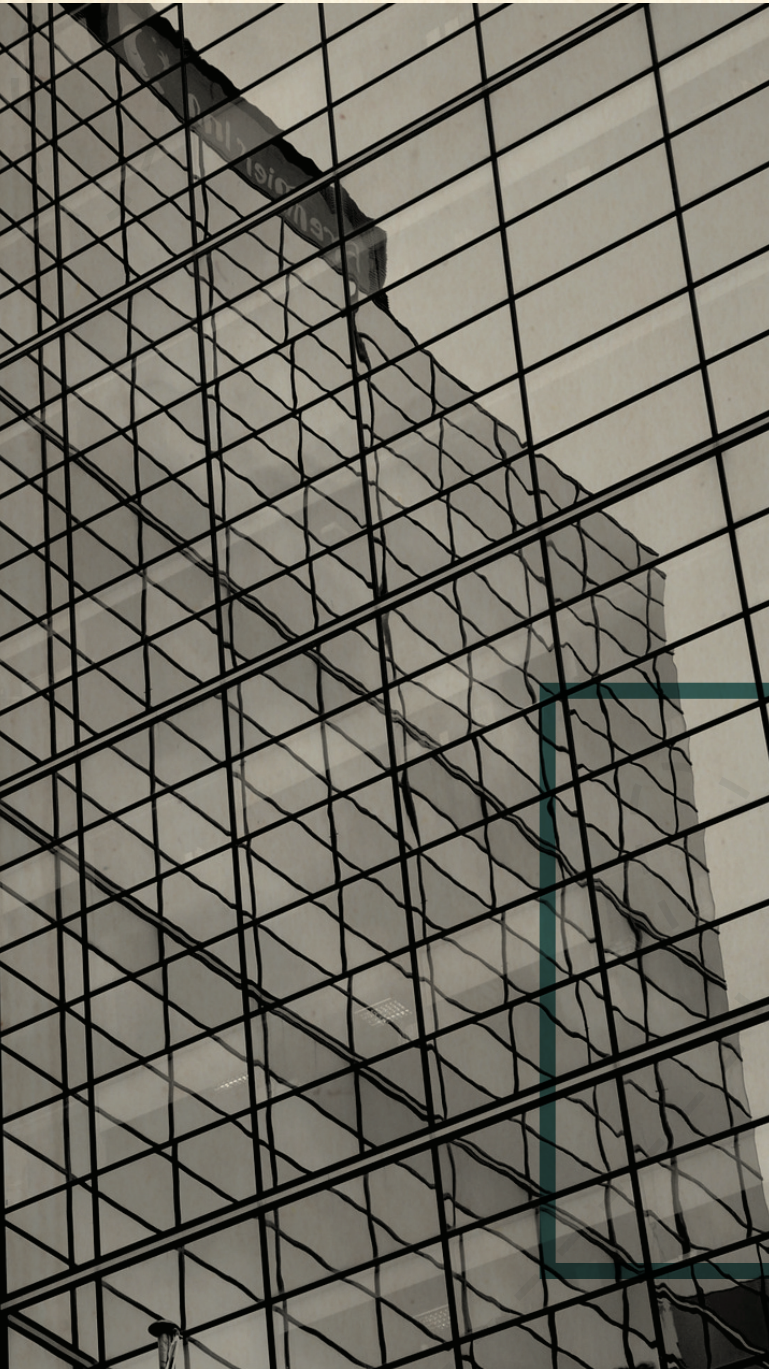
"The available data suggest that the risk from combined exposures to residues of pesticides with different modes of action is not appreciably greater than the risk from residues of the individual pesticides, when exposure is below the respective ADIs or ARfDs"¹⁰⁰.

This type of misuse of tools has blocked an efficient risk assessment for mixtures in food for over 10 years now (Regulation 396/2005 requires EFSA to present methods for mixture toxicity assessment), leaving European consumers unprotected against the adverse effects of toxic mixtures.

99. PAN report A poisonous Injection

100. www.efsa.europa.eu/en/efsajournal/pub/705.htm

4.4 AOP, another industry tool to disqualify observed adverse effects in animal studies?



What will happen with AOP at implementation time? An industry article (Patlewicz, RTP, 2015¹⁰¹) already speculates with great pleasure about the advantages for industry of AOP: *“For bypassing tier 1 tests? For bypassing in-vivo tests?”*.

The same goes on in the article of Boobis et al. ¹⁰², suggesting the use of AOPs to disqualify undesirable outcome of animal testing: *“Understanding of the mode of action of a chemical carcinogen will contribute to the consideration of the human relevance of the animal findings”*.

Given the potential conflict of interest, it is very important that these tools and especially the implementation of the tools is overseen by fully independent experts, experts who are not working for industry, not working for industry lobby groups, and have no connection whatsoever to industry or industry lobby groups, let alone financial ties. An open mind to current science should be the condition for every expert and no biased opinions present.

101. Grace Patlewicz, Ted W. Simon, J. Craig Rowlands, Robert A. Budinsky, Richard A. Becker, (2015). Proposing a scientific confidence framework to help support the application of adverse outcome pathways for regulatory purposes, *Regulatory Toxicology and Pharmacology* 71 463–477

102. Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D., Farland, W.. (2006). IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans. *Crit. Rev. Toxicol.* 36(10): 781–792.



Conflicts of interest

5.1 Matter of intentions

THE USE OR MISUSE of tools is just a matter of intentions. In almost all cases so far, industry didn't accept adverse effects observed in (their own) animal studies that might have a negative commercial effect of their own product¹⁰³. Industry commented that the effects were not relevant for humans, that they were within historical control ranges, were statistically insignificant, not-treatment related, species-specific, indirect, reversible, or even beneficial. Industry will fight an undesired outcome of an adverse effects that has a commercial impact. And this makes

sense since the mission of industry is to make profit and therefore it focuses on the scientific research that helps reach this mission. Science for industry is the version of scientific interpretation that supports profit.

This is exactly why the credibility of the public-private partnership AOP-program (especially the FP7 program SEURAT) can be questioned, as well as the meetings with the involvement of industry. They help to design a methodology that has the potential to be used and "misused".

103. PAN report Resubmission

A regulatory methodology should always be developed strictly by an independent scientific panel or working group, steered by government officials. Industry should not be given the opportunity to develop their own rules. Rules and methodology, as a matter of principle, should be made by totally independent experts focussing on the common good. Of course industry is interested in the methodology and they put a lot

of energy in advocating their views given the many industry opinions and meeting reports published in scientific journals. But they should only be allowed to do their advocacy work, commenting the outcome of government expert panels, in dedicated meetings together with other stakeholders in a balanced composition. For AOP, so far, we only note an unfair and unbalanced process, and science has been made subject to specific profit interests.

5.2 EU Joint Research Center (and OECD) uncritical towards industry

A JRC-invited-only meeting took place in 2011 in Ispra, Italy on toxicity pathways and AOP with BASF- Novartis- and DOW-employees together with people linked to industry lobby group ILSI, for which the financial support was provided by the American Chemistry Council (ACC)¹⁰⁴. Similarly, the article published on AOP in *Regulatory Toxicology and Pharmacology* (Tollefsen et al. RTP, 2014¹⁰⁵, Becker et al, 2015¹⁰⁶) is a result of a collaborative work of JRC and OECD representatives and DuPont-employees following another invited-only meeting that took place in Italy in 2014 with representatives of ACC,

Unilever and DuPont and sponsored by ACC, ECETOC and ILSI-HESI. Another meeting in Ispra, Italy (2014, Berggren¹⁰⁷) was sponsored by the Cosmetics Europe and attended by representatives of DOW, Unilever, P&G, L'Oreal and Henkel. JRC and OECD are clearly cooperating with industry on the development of the tool AOP. These are government officials collaborating with industry.

During a Paris OECD-meeting on AOP in 2014, 6 representatives of industry were present¹⁰⁸, and several of them were also invited to the JRC-invited-only meetings.

104. Maurice Whelan and Melvin Andersen, Toxicity Pathways – from concepts to application in chemical safety assessment, JRC, 2013

105. Knut Erik Tollefsen, Stefan Scholz, Mark T. Cronin, Stephen W. Edwards, Joop de Knecht, Kevin Crofton, Natalia Garcia-Reyero, Thomas Hartung, Andrew Worth, Grace Patlewicz, Applying Adverse Outcome Pathways (AOPs) to support Integrated Approaches to Testing and Assessment (IATA), *Regulatory Toxicology and Pharmacology* 70 (2014) 629–640

106. Richard A. Becker, Gerald T. Ankley, Stephen W. Edwards, Sean W. Kennedy, Igor Linkov, Bette Meek, Magdalini Sachana, Helmut Segner, Bart Van Der Burg, Daniel L. Villeneuve, Haruna Watanabe, Tara S. Barton-Maclaren (2015). Increasing scientific confidence in adverse outcome pathways: Application of tailored Bradford-Hill considerations for evaluating weight of evidence. *Regulatory Toxicology and Pharmacology* 72(3): 514-537

107. Elisabet Berggren, Patric Amcoff, Romualdo Benigni, Karen Blackburn, Edward Carney, Mark Cronin, Hubert Deluyker, Françoise Gautier, Richard S. Judson, Georges E.N. Kass, Detlef Keller, Derek Knight, Werner Lilienblum, Catherine Mahony, Ivan Rusyn, Terry Schultz, Michael Schwarz, Gerrit Schürmann, Andrew White, Julien Burton, Alfonso M. Lostia, Sharon Munn, and Andrew Worth, *Chemical Safety Assessment Using Read-Across: Assessing the Use of Novel Testing Methods to Strengthen the Evidence Base for Decision Making*, volume 123 | number 12 | December 2015 • *Environmental Health Perspectives*

108. OECD Headquarters, Paris, 12-13 June 2014, SUMMARY REPORT OF THE 7TH MEETING OF THE EXTENDED ADVISORY GROUP ON MOLECULAR SCREENING AND TOXICOGENOMICS, ENV/JM/TG/M(2014)4

It is worrying to note that experts from JRC and UK/NL/DE-national institutes on top of this are part of industry technical HESI (Health and Environmental Sciences Institute)-committees¹⁰⁹ -another ILSI-network “lobby” group- on similar topics. Government expert participation in these committees and meetings allows HESI to make claims of an alleged scientific consensus while none actually exists¹¹⁰. This shows that government officials should keep a distance from the industry and should participate only in meetings with a balanced stakeholder representation (from industry and civil society).

From some distance it looks like if a ‘network’ of generally the same experts is operating, dominating the development of AOP at all levels, which is an inner circle, excluding the public, other stakeholders and independent scientists. Promoters of a tool, implement the tool in an “independent” way. A déjà vu¹¹¹.

109. <http://hesiglobal.org/animal-alternatives-in-environmental-risk-assessment/>

110. R.Steinzor and W.Radin, Cozying up, Center of Progressive Reform, 2012

111. PAN Europe report on EFSA and TTC (Threshold of Toxicological Concern), 2011. “A toxic Mixture?” www.pan-europe.info/resources/reports

5.3 Science is the victim

On the JRC-website it is stated: *"As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support.."*. But nonetheless the JRC-work on AOP is clearly performed in collaboration with the industry and therefore not in such an independent way.

On the OECD-website we read: *"We recommend policies designed to improve the quality of people's lives"*. OECD-policies, therefore, should be based on current insights of science and technology, protect people and the environment and not allow certain interests to do their advocacy work in constructing rules for themselves as is the case of AOP.

It is hard to understand why industry is allowed to be part of the development of AOP. The main mission of industry is to serve their shareholders and especially to generate profit. This is a clear conflict of interest with governments who have to serve and protect their citizens and provide them a healthy environment. There is little doubt about the fact that any industry involvement in the development will have elements of industry advocacy and this is unwanted. Industry should not be able to influence its own regulatory measures.



5.4 Are there indications of obvious industry misuse already?

The fragrance-industry is already using the predictive tools on a full scale. Is this what we can expect once AOP is used in regulatory decisions? An example of this approach is highlighted below:

A study on the fragrance chemical linalyl isobutyrate (study DeKant and others¹¹²) gives a flavour of what one can expect from the 'self-regulation' by industry itself on non-animal testing. Can the substance cause chromosome aberrations? There are no data and therefore the information is taken from linalyl isobutyrate. Does the substance have clastogenic activity? There are no data and the information is taken from another structurally related chemical, linalyl acetate. What about developmental toxicity? There are no data, and the information is taken from again another chemical, linalool. Reproductive toxicity? No data and the information is taken from chemical nr. 4, dehydrolinalool. Is there a no effect level (NOAEL)? Not available for the substance, but the exposure of 26 ppb is below the TTC (a tool to predict the probability of safety) of 30 ppb. DNA-binding, mutagenicity, genotoxicity alerts, all were estimated using a QSAR toolbox. There are no invitro studies applied to connect to a certain MoA to the structurally related chemicals, the industry just bases its conclusions on assumed similarities.

112. Api AM, Belsito D, Bhatia S, Bruze M, Calow P, Dagli ML, Dekant W et al (2015). RIFM fragrance ingredient safety assessment, linalyl isobutyrate, CAS registry number 78-35-3. Food Chem Toxicol. 84:S76-87

This approach based on read-across can be characterised as non fact-based and includes the danger of shopping around to get a desired outcome. There is no mechanistic information on the substance itself, no biological key events, no in-vitro test linked to this key event, and nothing to link mechanistic information to the apical endpoint.

This is the approach we must avoid in order to limit the number of “false negatives” from regulatory assessment of chemicals and the release of potentially dangerous substances to the environment.

A report of Women’s voices on the Earth¹¹³ on the ‘self-regulation’ of the fragrance-industry gives a picture of what the agenda of industry is. The assessments published by RIFM, the Research Institute of Fragrance Materials, use an early version of AOP with emphasis on QSAR and ‘read-across’. The report of Woman’s voices concludes that

“Most of the basic science studies on fragrance ingredients are conducted by the manufacturers themselves and have never been published in a peer-reviewed scientific journal. There is no independent review of laboratory practices, appropriate controls, levels of significance or any of the hallmarks of authoritative science, to ensure that the results of these studies have not been manipulated to serve the interests of the manufacturer conducting the testing”. And concludes that “the European Commission Scientific Committee on Consumer Safety (SCCS) reviewed studies on fragrance materials submitted by RIFM, to produce their opinions on the safety of certain fragrance materials. Their assessments of RIFM studies commonly noted the studies’ scientific inadequacies, such as incomplete data, inability to confirm identify of the test substance, invalid test protocols, lack of appropriate controls, and more. The SCCS frequently commented that the data submitted could not reliably be used to form a conclusion of safety”.

Industry itself on ‘read-across’ writes¹¹⁴:

“...after chemical similarity has been established, the availability of high quality biological activity (e.g., toxicity) data is fundamental to the read-across prediction”

and disqualifies the approach of RIFM to solely rely on chemical similarities.

113. A. Scranton, Unpacking the Fragrance Industry: Policy Failures, the Trade Secret Myth and Public Health, An investigative report by Women’s Voices for the Earth November 2015

114. Nicholas Ball, Mark T. D. Cronin, Jie Shen, Karen Blackburn, Ewan D. Booth, Mounir Bouhifd, Elizabeth Donley, Laura Egnash, Charles Hastings, Daland R. Juberg, Andre Kleensang, Nicole Kleinstreuer, E. Dinant Kroese, Adam C. Lee, Thomas Luechtefeld, Alexandra Maertens, Sue Marty, Jorge M. Naciff, Jessica Palmer, David Pamies, Mike Penman, Andrea-Nicole Richarz, Daniel P. Russo, Sharon B. Stuard, Grace Patlewicz, Bennard van Ravenzwaay, Shengde Wu, Hao Zhu and Thomas Hartung, Toward Good Read-Across Practice (GRAP) Guidance, ALTEX 33(2), 2016, <http://dx.doi.org/10.14573/altex.1601251>

5.5 What history tells us

The cigarette industry is most known for hiding the truth¹¹⁵ about cancer and the addiction of their products and could only be stopped after several US states sued them and they had to pay for the damage. This only happened after 40 years of denial and successful litigation of the cigarette industry.

On pharmaceuticals a similar story can be told. Ben Goldacre wrote his famous book "Bad Pharma"¹¹⁶ showing manipulation with trials:

"New drugs are tested by the companies that make them, often in trials designed to make the drug look good, which are then written up and published in medical journals. Unless, that is, the company doesn't like the result of the trial (maybe it shows the drug not working or having severe side-effects), in which case this result might be hidden".

This shows the major historical mistake to ask pharmaceutical companies to test their own products.

Can we expect that chemical companies are different? There are a range of fraudulent cases on testing pesticides in laboratories like IBT¹¹⁷, Craven and others. While regulators feel that imposing GLP-certificates for laboratories provides a guarantee for the quality of laboratory, it doesn't provide a guarantee against fraud and quality of data.

Next to fraud, there are many other ways to change conclusions of testing. Undesirable testing results of course can simply not be published. Since industry claims confidentiality of all their test reports (only a summary is published), the truth is not easily revealed.

Many times, however, when independent scientists observe harm from chemicals and start publishing about it, industry tries to counterbalance this by publishing their own studies. For example, Lesser et al¹¹⁸, looked at articles on the health effects of various soft drinks. The proportion of studies with unfavorable conclusions was 0% for all industry funding versus 37% for no industry funding. The same happened on the pesticide Atrazine, where endocrine disrupting properties of Atrazine as demonstrated by Hayes¹¹⁹ were unfairly countered by industry. It is clear that the outcome of industry studies should always be considered with caution and always balanced against independent research.

115. https://en.wikipedia.org/wiki/Tobacco_industry

116. <http://www.theguardian.com/books/2012/oct/17/bad-pharma-ben-goldacre-review>

117. https://en.wikipedia.org/wiki/Industrial_Bio-Test_Laboratories

118. Lenard I. Lesser, Cara B. Ebbeling, Merrill Goozner, David Wypij, David S. Ludwig, Relationship between Funding Source and Conclusion among Nutrition-Related Scientific Articles, PLoS Medicine | www.plosmedicine.org January 2007 | Volume 4 | Issue 1 | e5

119. TYRONE B. HAYES, There Is No Denying This: Defusing the Confusion about Atrazine, 1138 BioScience • December 2004 / Vol. 54 No. 12

6

Conclusions

6.1 Conflicts of interest

There are big concerns with the extensive and non-balanced involvement of industry groups in the development of the AOP-tool. Tools such as the Adverse Outcome Pathway Knowledge Base serve as guidance to OECD countries to regulate chemicals; hence they should be developed and applied by regulatory bodies without the influence of the chemical industry organisations that are to be regulated and by experts that have no links to industry whatsoever. In the SEURAT-program and at the OECD this is not the case.

The SEURAT program is even dominated by industry and industry lobby groups like ILSI¹²⁰ that runs one of the six elements of SEURAT, the program COSMOS. In COSMOS a range of old industry lobby ideas are warmed up and revised for regulatory use. Industry, especially ILSI, is promoting for a long time to make considerations on 'mode of action' (MoA) the central element of risk assessment¹²¹, based on older ideas from the US¹²². Subsequently the MoA approach was promoted by ILSI and ILSI-linked experts in WHO^{123, 124} (for cumulative toxicity) and EU Food Authority EFSA¹²⁵. Prof. Boobis, a UK professor, serves for years as the chair of the Board of Trustees of ILSI and has -according to his declaration of interest at EFSA- offered many consultancy services for industry. Nevertheless, he managed to be a member of the EFSA panels for years and is a member of WHO/IPCS working groups. Dr. Meek, a Canadian expert, is also connected to ILSI and publishes mainly with industry experts. Meek was included in SEURAT as academic scientist. Real independent academic scientists generally are a minority in panels and working groups.

6.2 Potential misuse of the AOP tool

The conflicts of interest of industry groups are clear: while mechanistic data is useful, risk assessment tools should not be used to restrict toxicological investigation. If an AOP-tool would be applied now, the general lack of knowledge about mechanisms of action would mostly lead to assumptions and speculation about AOPs. Speculations for instance about the differences between AOPs and observed mechanism/effects in different animal studies, and what should be considered the "real" effect. The (arbitrary) conclusion on the "real" mechanism/effect might next be used to question animal toxicity studies showing adverse effects and ultimately even lead to disqualifying the

120. ILSI, International Life Science Institute, an industry lobby group

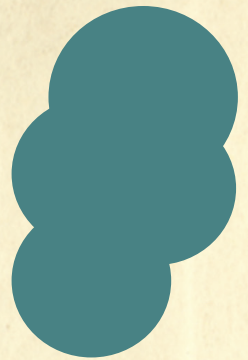
121. Neil Carmichael, Melanie Bausen, Alan R. Boobis, Samuel M. Cohen, Michelle Embry, Claudia Frujtier-Pöloth, Helmut Greim, Richard Lewis, M.E. (Bette) Meek, Howard Mellor, Carolyn Vickers, and John Doe, Using mode of action information to improve regulatory decision-making: An ECETOC/ILSI RF/HESI workshop overview, *Critical Reviews in Toxicology*, 2011; 41(3): 175–186

122. C. Sonich-Mullin, R. Fielder, J. Wiltse, K. Baetcke, J. Dempsey, P. Fenner-Crisp, D. Grant, M. Hartley, A. Knaap, D. Kroese, I. Mangelsdorf, E. Meek, J. M. Rice, and M. Younes, IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis, *Regulatory Toxicology and Pharmacology* 34, 146–152 (2001)

123. M. E. Meek, A. Boobis, I. Cote, V. Dellarco, G. Fotakis, S. Munn, J. Seed and C. Vickers, New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis, *J. of Applied Toxicology*, Oct. 2013.

124. M.E. (Bette) Meek, Alan R. Boobis, Kevin M. Crofton, Gerhard Heinemeyer, Marcel Van Raaij, Carolyn Vickers, Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework, *Regulatory Toxicology and Pharmacology* 60 (2011) S1–S14

125. PAN report A Poisonous injection



adverse effects observed in actual testing. While generally more mechanistic information based on actual testing would be helpful, AOP based on assumptions, read-across and speculation could create a suggestion of safety and undermine regulatory action based on the precautionary principle, particularly in contentious debates where there is high scientific uncertainty and/or disagreement about desired outcomes and societal considerations are prominent.

Industry has failed its responsibility to test chemicals before putting them on the market, increasing their profits while moving the risks and costs to society; it is a bit strange to note now that the same industry is saying that testing is too costly and we have to move to AOP.

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ANIMAL
TESTING?

NO, too
expensive.

THEN MODE
OF ACTION?

NO,
we have not much knowledge
about modes of action

SO WHAT?

We use our own "expert judgement",
prediction, speculation and
assumption, this is the cheapest
option, and we call it AOP.

6.3 Recommendations

The analysis on AOP and the conclusions lead to the following recommendations:

1.



AOPs are a relevant research topic

AOP should only be used as a priority-setting tool for chemicals with unknown toxicity and used to implement the precautionary principle



2.

3.



The content of AOPs should give priority to effects on the foetus and always include this vulnerable phase in any AOP

AOPs should give a priority to “mixture toxicity” and every AOP should be developed taking this into account



4.

5.



AOPs are not ready for use as long as a solution for mixture toxicity is not included

AOPs are not ready for use in risk assessment in the EU for the foreseeable future because the level of predictability is unknown



6.

7.



Full independent 'audits' of the factual basis and databases for elements of AOP are necessary, including verification of the effectiveness and the level of predictability

AOPs should only be used to fill data gaps and never be (mis)used in cases of data-rich chemicals such as pesticides and biocides



8.

9.



AOPs should never be used to overrule toxicity data from experimental studies

Use of an AOP as a prediction tool (for cosmetic chemicals) should be based on robust data and strict guidelines, and any 'partial' or 'qualitative' AOP disregarded



10.

11.



AOP should be developed and implemented by fully independent scientists and experts; commercial interested parties should only be allowed to have a stakeholder role in balance with other stakeholders

Current scientific insights and scientific data on elements like low-dose effects and non-monotonic dose-response effects should be included while the application of thresholds should be abandoned, unless scientifically proven



12.

The reason for chemical testing is their potential toxicity following human and environmental exposure; a simple way to reduce animal testing is to reduce the production of toxic chemicals. This has not been proposed by the industry.