Integration of epidemiological findings with mechanistic evidence in regulatory pesticide risk assessment: EFSA experiences

Pelkonen, Olavi; Bennekou, Susanne Hougaard; Crivellente, Federica; Terron, Andrea; Hernandez, Antonio F.

Published in:
Archives of Toxicology

Link to article, DOI:
[10.1007/s00204-019-02467-w](https://doi.org/10.1007/s00204-019-02467-w)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Pelkonen, O., Bennekou, S. H., Crivellente, F., Terron, A., & Hernandez, A. F. (2019). Integration of epidemiological findings with mechanistic evidence in regulatory pesticide risk assessment: EFSA experiences. *Archives of Toxicology*, 93(6), 1779-1788. <https://doi.org/10.1007/s00204-019-02467-w>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Archives of Toxicology

Integration of epidemiological findings with mechanistic evidence in regulatory pesticide risk assessment – EFSA experiences --Manuscript Draft--

Manuscript Number:	
Full Title:	Integration of epidemiological findings with mechanistic evidence in regulatory pesticide risk assessment – EFSA experiences
Article Type:	Meeting Report
Corresponding Author:	Olavi Pelkonen University of Oulu Oulu, FINLAND
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Oulu
Corresponding Author's Secondary Institution:	
First Author:	Olavi Pelkonen
First Author Secondary Information:	
Order of Authors:	Olavi Pelkonen Susanne H Bennekou Federica Crivellente Andrea Terron Antonio F Hernandez
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Toxicological risk assessment of plant protection products (PPP) is currently carried out with the principal input from regulatory toxicology studies following OECD test guidelines, with little input from epidemiological data. An EFSA-commissioned systematic review of pesticide epidemiological studies (Ntzani et al 2013) revealed statistically significant associations, among others, between pesticide exposures and Parkinson's disease and childhood leukaemia. Thereafter, EFSA launched a project with a mandate for the Plant Protection Products and their residues (PPR) Panel to set the ground for the use of epidemiological data in the risk assessment of pesticides, as requested by Regulation (EC) 1107/2009. The project culminated with the publication of two EFSA's scientific opinions on the potential contribution of experimental investigations and epidemiological studies in PPP risk assessment and with the scientific conference held on 20 November, 2017, in Parma, Italy. The application of modern methodologies in exposure assessment, toxicology and epidemiology would improve the pesticide risk assessment process and support a mechanistic shift for the integration of these three disciplines under a novel paradigm in risk assessment. The application of the Adverse Outcome Pathways (AOP) conceptual framework to this approach would contribute to gain insight into the biological plausibility of a hazard identified in epidemiological or experimental studies and would inform an Integrated Approach to Testing and Assessment (IATA) within a regulatory context.
Suggested Reviewers:	

[Click here to view linked References](#)

Final Version 190329

Meeting Report¹

Integration of epidemiological findings with mechanistic evidence in regulatory pesticide risk assessment – EFSA experiences

Olavi Pelkonen¹, Susanne H. Bennekou², Federica Crivellente³, Andrea Terron³, Antonio F. Hernandez⁴

¹University of Oulu, Research Unit of Biomedicine/Pharmacology and Toxicology, Oulu, Finland

²National Food Institute, Division for Risk Assessment and Nutrition, Technical University of Denmark

³EFSA, European Food Safety Authority, Parma, Italy

⁴University of Granada School of Medicine, Granada, Spain

Correspondence:

Olavi Pelkonen, University of Oulu, Research Unit of Biomedicine/Pharmacology and Toxicology, Aapistie 5 B, FIN-90014 Oulu (Finland); e-mail: olavi.pelkonen@oulu.fi; mobile: +358405075196

¹ This report is based on plenary talks and extensive discussions at the EFSA Scientific conference on the use of epidemiological findings in regulatory pesticide risk assessment on 21 November, 2017, in Parma, Italy. The speakers were Federica Crivellente (EFSA), Susanne Hougaard Bennekou (Danish EPA, EFSA PPR Panel), Bette Meek (University of Ottawa), Antonio Hernandez Jerez (University of Granada, EFSA PPR Panel), David Miller (US-EPA), Karin Angeli (ANSES), Laura Beane Freeman (US NCI), Judy Lakind (LaKind Associates), Marie-Odile Rambourg (ANSES), Manolis Kogevinas (ISGlobal), Carol Burns (ECPA), Martin Dermine (PAN Europe). The speakers and discussants are thanked for their contributions during the meeting; however, report authors are responsible for views and recommendations expressed in this report (see the EFSA site: <https://www.efsa.europa.eu/en/events/event/171121-0>).

Abstract

Toxicological risk assessment of plant protection products (PPP) is currently carried out with the principal input from regulatory toxicology studies following OECD test guidelines, with little input from epidemiological data. An EFSA-commissioned systematic review of pesticide epidemiological studies (Ntzani et al 2013) revealed statistically significant associations, among others, between pesticide exposures and Parkinson's disease and childhood leukaemia. Thereafter, EFSA launched a project with a mandate for the Plant Protection Products and their residues (PPR) Panel to set the ground for the use of epidemiological data in the risk assessment of pesticides, as requested by Regulation (EC) 1107/2009. The project culminated with the publication of two EFSA's scientific opinions on the potential contribution of experimental investigations and epidemiological studies in PPP risk assessment and with the scientific conference held on 20 November, 2017, in Parma, Italy. The application of modern methodologies in exposure assessment, toxicology and epidemiology would improve the pesticide risk assessment process and support a mechanistic shift for the integration of these three disciplines under a novel paradigm in risk assessment. The application of the Adverse Outcome Pathways (AOP) conceptual framework to this approach would contribute to gain insight into the biological plausibility of a hazard identified in epidemiological or experimental studies and would inform an Integrated Approach to Testing and Assessment (IATA) within a regulatory context.

Key words: plant protection products, pesticides, risk assessment, epidemiology, adverse outcome pathway (AOP), exposure, Integrated Approach to Testing and Assessment (IATA)

Introduction

According to the current European Union (EU) legislation on the placing of plant protection products (PPP) on the market, epidemiological studies are of particular value and must be submitted ‘where available, and supported with data on levels and duration of exposure, and conducted in accordance with recognised standards’ (Regulation No. 1107/2009). Likewise, Regulation No. 283/2013, concerning the data requirements for active substances (AS), laid down that ‘relevant epidemiological studies shall be submitted, where available’, but there is ‘no obligation for the petitioners to conduct epidemiological studies for the AS undergoing the approval or renewal process’. However, a systematic literature review is required for the AS and its relevant metabolites, although this is not restricted to human observational studies but should also include experimental studies published in the open literature.

Epidemiological data on a specific pesticide exposure has not been submitted to the regulatory authority at first approval and only occasionally they are provided at the time of renewal of an AS and consequently has rarely contributed to the risk assessment process thus far. Nevertheless, several epidemiological studies and meta-analyses are available in the scientific literature; though despite the large amount of epidemiological studies reporting associations between pesticide exposure and human health outcomes, the impact of such studies in regulatory risk assessment is still limited.

EFSA commissioned to University of Ioannina (Greece) a systematic literature review and meta-analyses of epidemiological studies published from 2006 to 2012 for surveying potential associations between pesticide exposure and a wide array of human adverse health outcomes (Ntzani et al 2013). Although statistically significant associations were found for some diseases (liver cancer, breast cancer, stomach cancer, amyotrophic lateral sclerosis, asthma, type II diabetes, childhood leukaemia and Parkinson’s disease), no firm conclusions could be drawn for the majority of them. Furthermore, the report alluded that the epidemiological studies reviewed suffered from a number of limitations and large heterogeneity of data, including broad pesticides definitions (and therefore inaccurate pesticide exposure estimates) and consequently the scope of the report did not allow drawing in-depth associations between pesticide exposure and specific health outcomes.

On the basis of the external report, in 2013 EFSA initiated a Pesticide Epidemiology project, which started with a “Stakeholder Workshop on the use of epidemiological findings in regulatory pesticide risk assessment” held on 18 February 2015 in Paris. The project

culminated four years later in the publication of two scientific opinions: **Scientific Opinion** on the Investigation into Experimental Toxicological Properties of plant protection products (PPPs) Having a Potential Link to Parkinson’s Disease and Childhood Leukaemia (EFSA PPR Panel, 2017a), and **Scientific Opinion** of the PPR Panel on the follow-up of the findings of the External Scientific Report “Literature review of epidemiological studies linking exposure to pesticides and health effects” (EFSA PPR Panel, 2017b). The two Scientific Opinion were also presented and debated at the EFSA Conference on epidemiology on 20 November, 2017, in Parma, Italy. Additional initiatives are currently ongoing in EFSA with the recognition that epidemiology is an overarching item for EFSA and as such will be led by the Scientific Committee.

This paper aims to raise awareness of the scientific community about this initiative by summarizing the results and recommendations of the above mentioned EFSA project, i.e. search ways to integrate experimental, epidemiological and regulatory approaches for pesticide risk assessment. Additional details of the project can be found at the EFSA website (<https://www.efsa.europa.eu/en/news/62081>). A scheme of the project is presented in Figure 1.

Epidemiological studies – role in pesticide risk assessment and room for improvements

The Scientific Opinion (EFSA PPR Panel, 2017b) proposed a methodological approach specific for pesticide ASs to make appropriate use of epidemiological data for risk assessment. The approach should include the analysis of strengths and weaknesses of epidemiological studies after the appropriate quality considerations as well as the investigation of biological plausibility of the epidemiological associations (Figure 2).

Major limitations in current pesticide epidemiological studies

The systematic appraisal of the epidemiological evidence allows a number of methodological limitations to be identified. These limitations prevent robust conclusions to be drawn, and they include, but are not limited to: a) less than optimal study designs, as most of studies are case-control and cross-sectional studies, which lack temporal concordance. Besides, many studies are not sufficiently powered; b) use of broad definition of exposure assessed through questionnaires (often not validated) and seldom by biomarkers of exposure in biological matrices. Besides, information on exposure to individual pesticides is scarce and, where available, very often it is not quantitatively reported; c) deficiencies in outcome assessment

(broad outcome definitions and use of self-reported outcomes or surrogate outcomes); d) deficiencies in reporting, confounder control and statistical analysis (including multiple testing); f) selective reporting of results and publication bias.

Pesticide exposure data in environmental epidemiology: limitations and quality assessments

There are large methodological difficulties in assessing and measuring exposure to pesticides in relation to epidemiological investigations. Human pesticide exposures are most of the time complex, involving many active substances, co-formulants and other ingredients. Exposures can be occupational (applicators and farmworkers), para-occupational (by-standers) or residential; and may be acute (as a result of exposure to high doses), or chronic, that is long-term low-dose (as a result of intermittent, irregular, but usually highly variable exposure with respect to time and intensity). Pesticide exposure may be measured by environmental analyses, personal exposure monitoring or by monitoring of human material (e.g., blood, urine, hair...). Alternatively, exposure can be modelled using job-exposure or crop-exposure matrices, geo-coding residential addresses, etc. Because of all these complexities, exposure misclassification occurs to a large extent in pesticide epidemiology such that the possibility to detect an adverse effect associated to a specific AS is less likely. Preferentially exposure assessments should be designed for specific situations depending on specific hypotheses, outcomes, timing, intensity etc. In any case, improvements in exposure assessment are critically important because there is relatively convincing knowledge about the health risk effects of pesticide exposures.

Because of the increasing interest in using epidemiology for regulatory decision-making, there is obviously a growing demand for high quality exposure data. Methodological limitations of individual studies make meaningful weight of the evidence assessment difficult. Transparent and systematic instruments are in demand for use for both study design and quality assessment, as well as to help to address problems in exposure assessment. One such tool is Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument (LaKind et al 2014; Goodman et al 2017), which is in use in USA and Europe. Exposure quality evaluation according to this instrument regarding especially biomarker development includes the following elements: exposure and biological relevance, specificity, method sensitivity, contamination, stability, method requirements, adjust for matrix dilution, temporality, variability/misclassification, and general epidemiological study design considerations. A recent review stresses the importance of multiple biomonitoring samples

collected over a period of toxicological relevance and with consideration of exposure patterns (LaKind et al 2019).

Reliability and relevance of epidemiological studies

The Scientific Opinion (EFSA PPR Panel, 2017b) also focused on matters which would enhance the quality and relevance of epidemiological research on pesticides for risk assessment purposes, such as 1/ adequate assessment of exposure at individual pesticide level to minimize exposure misclassification, 2/ valid and reliable outcome assessment, 3/ accounting for potential confounders and 4/ adequate statistical analysis and reporting of results. Furthermore, systematic reviews and meta-analyses of the available epidemiological evidence can provide a useful approach for hazard identification as these tools allow generation of summary data, increase the statistical power and precision of risk estimates by combining the results of all individual studies (e.g, Moher et al, 2015; Shamseer et al, 2015). The crucial goal is the integration of epidemiological and toxicological data in the process of hazard identification/characterization and weighting the evidence from different sources, e.g. observational, *in vivo*, *in vitro* and *in silico* studies (Hernandez and Tsatsakis, 2017). The reliability, relevance and consistency of single studies and pooled evidence should be considered for a weight of evidence approach. This, together with all available data, will be used in an integrated approach to testing and assessment (IATA) where the available mechanistic data will lend support to the development of appropriate Adverse Outcome Pathway (AOP); AOP informed IATA will therefore contribute to pesticide risk assessment. Conclusions as to the role of epidemiological evidence in pesticide risk assessment included the following points: 1/ Current epidemiological studies can be useful for hazard identification/characterization of pesticides. 2/ Better designed epidemiological studies may improve risk assessment of pesticides. 3/ In this connection, it is important to stress that the assessment of exposure represents one of the most relevant limitations with the epidemiological studies carried out with pesticides. 4/ Biological/mechanistic plausibility supports associations between pesticide exposure and the adverse outcomes described in human epidemiological studies, including complex diseases that are unlikely captured by *in-vivo* experimental toxicological studies. 5/ AOP and mode of action (MoA) frameworks should be used to link the outcome from epidemiological studies in order to weight their conclusions and establish a mechanistic biologically plausible link between the AO and the experimental studies, and finally, 6/ integration of all these scientific evidence in a structured dose and temporal concordant framework would benefit from moving to a mechanistic-based

1 risk assessment able to contribute to the identification of risk factors relevant to human
2 diseases.

3
4 A point of disagreement among some stakeholders has been a question of all-inclusiveness of
5 studies to assessment vs. quality-assessment of epidemiological evidence. Scientifically it is
6 clear that quality of studies is a major determinant and it is not possible to base regulatory
7 decisions on poor epidemiology data. Even good epidemiological studies may have very
8 limited or no weight in the final assessment, if appropriate data is lacking or insufficient (e.g.
9 data on specific pesticides under assessment). Still, with quality epidemiological studies
10 important questions remain to be considered, for example: 1/ How can the regulatory process
11 ensure optimal timing between the re-assessment process of AS and the availability of
12 appropriate epidemiological studies, 2/ What is the relevance of negative results in risk
13 assessment? (which relates to transparency of reporting the results), and 3/ Can dose-response
14 data from epidemiological studies, if available, be used to identify a point of departure level
15 suitable for benchmark-dose modelling, 4/ What kind of findings will trigger the adoption of
16 precautionary measures in risk assessment as stipulated by the regulation? These are all very
17 valid questions which have to be dealt with when integrating the different lines of evidence,
18 i.e. epidemiology with experimental research.

19 *The US- Environmental Protection Agency (EPA) approach*

20
21 In the USA, the Environmental Protection Agency/Office of Pesticide Programs (EPA/OPP)
22 has the central role in pesticide risk assessment due to its regulatory mandate. EPA/OPP is
23 increasingly considering on-going epidemiological studies, and the collection and use of
24 incident data for pesticide risk assessment. The main regulatory tool for this purpose is the
25 OPP Framework for incorporating epidemiology and incident data (US-EPA 2016). EPA/OPP
26 intends to make increasing use of these data for human health risk assessment under the most
27 scientifically robust and transparent way. The guiding principles of the framework include the
28 use of epidemiology reviews in a tiered process in problem formulation, the identification of
29 major factors that will inform risk assessment, and MoA/AOP Framework to identify key
30 events along a causal pathway where different sources of information (from experimental to
31 observational studies) can be organized and integrated. The key issues of the OPP Framework
32 are the assessments of exposure, health outcomes, confounding, statistical analysis and risk of
33 bias of individual epidemiological studies. OPP has adopted a tiered review assessment
34 approach to fulfil its regulatory mandate and respond to emerging public health issues,
35 manage program workload and prioritize potential risk issues that warrant systematic

1 investigation. Under this approach, each tier considers the usefulness of the assessment for its
2 intended purpose in order to ensure that the assessment produced is suitable and useful for
3 informing the needed decisions. Overall, concepts in EPA/OPP framework are similar in
4 many ways to EFSA's proposed framework, although also some differences exist because of
5 the different legal requirements (EFSA PPR Panel, 2017b).
6
7

8 9 *Agricultural cohort studies as key sources of pesticide epidemiological evidence*

10
11 One of the main sources of pesticide epidemiological findings is the US Agricultural Health
12 Study (AHS), a federally funded study that evaluates associations between pesticide
13 exposures and cancer and other health outcomes. The main features of the AHS studies
14 include a more informative study design (prospective cohort), improved exposure assessment
15 (self-reported, but ascertained in multiple ways and algorithms developed), a considerable
16 number of study subjects (~57000 applicators, ~32000 spouses), a more precise outcome
17 assessment (cancer registries, others self-reported, but ascertained by medical reports),
18 approach to etiologic analyses, and sub-studies for specific hypotheses. The project has
19 already resulted in numerous publications and it will continue, in some cases updating
20 previous findings on specific exposures and health outcomes (Andreotti et al., 2018). Further
21 details could be found at the AHS internet site (Ref: www.aghealth.nih.gov).
22
23

24
25 AGRICOH is a consortium of agricultural cohort studies from five continents (e.g., AHS,
26 French Agriculture and Cancer Study (AGRICAN), Cancer in the Norwegian Agricultural
27 Population (CNAP), etc.) initiated by the US National Cancer Institute and the International
28 Agency for Research on Cancer (IARC) in October 2010. The aim is to encourage and
29 support data pooling to study disease-exposure associations that individual cohorts do not
30 have sufficient statistical power to study. Cohorts participating in AGRICOH study involve
31 health outcomes in relation to environmental and occupational exposures in agricultural
32 settings (Ref; agricoh.iarc.fr).
33
34

35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 **Contribution of vigilance data to the risk assessment of pesticides**

51
52 Vigilance (surveillance and monitoring) systems include foremost activities related to the
53 detection, assessment, understanding and prevention of adverse events. Currently there are
54 systems able to detect pesticide-related incidences, such as work-related disease surveillance
55 systems, occupational disease registries, post-marketing surveillance programmes, non-
56 specific recording systems such as Poison Control Centres (PCCs), and EU alerting system on
57
58
59
60
61
62
63
64
65

chemical hazards (RASCHEM). However, there is considerable heterogeneity within and between EU member states regarding methodology for collection of vigilance data and furthermore, current schemes are not specifically designed for pesticides, resulting in e.g. poor data on exposures.

Several EU regulations require the notification, collection and/or reporting of pesticide-related adverse events in humans after acute or chronic exposures occurring in the work place, accidental or deliberate poisonings, etc. These include: a) EC Regulation 1107/2009, which requires that the authorisation holder shall record and report all suspected adverse reactions in humans, animals and the environment related to the use of the PPP; b) Directive 128/2009/EC for the sustainable use of pesticides requires that Member States shall put in place systems for gathering information on pesticide acute poisoning incidents, as well as chronic poisoning developments where available, among groups that may be exposed regularly to pesticides such as operators, agricultural workers or persons living close to pesticide application areas. However, a strategic guidance document on monitoring and surveying of impacts of pesticide use on human health and the environment has not yet been produced.

Therefore, considerable variability and uncertainties in the accuracy of information, exposure estimates and the assessment of causal relationship between exposure and adverse effects are at least partially due to lack of harmonisation. Development of an EU-wide vigilance framework for pesticides together with harmonisation of human incident data collection activities at the EU level and development of a valid method for assessing the weight/strength of the causal relationship ('imputability') for acute (and chronic) incidents are suggested as potential improvements for pesticide risk assessment (EFSA PPR Panel, 2017b, SAPEA 2018, Scientific Advice Mechanism 2018). A proposal for integrating vigilance into a process of the European Pesticide regulation can also be found in SAPEA (2018).

Use of the AOP framework to improve the utilization of epidemiological findings for pesticide risk assessment

The AOP concept is becoming a practical and pragmatic tool in toxicological research and regulatory risk assessment (Delrue et al 2024; Edwards et al 2016; Sakuratani et al 2018; Vinken 2018). The development of specific AOPs for parkinsonian motor symptoms and infant leukaemia as adverse outcomes was the principal objective of the EFSA PPR Panel to set the biological plausibility of the epidemiological associations found between exposure to

pesticides and the risk of developing Parkinson's disease (PD), and infant and childhood leukemia (EFSA PPR Panel, 2017a). Both diseases are complex entities and the first decision was the use specific symptoms (motor disturbances for PD) or biologically distinct entities (infant leukaemia or childhood leukaemia) as starting adverse outcomes (AOs) to develop the corresponding AOPs.

It is also of importance to note that motor disturbances could basically be captured by existing guidelines as clinical signs in both the standard repeat dose studies (OECD TG 408) as well as those developed for the study of neurotoxicity in adult and young laboratory animals (OECD TG 424) and the guideline for developmental neurotoxicity (OECD TG 426). However, degeneration of dopaminergic neurons at substantia nigra is not specifically covered by these guideline studies. Likewise, infant/childhood leukaemia are difficult to capture by the current regulatory testing paradigm used for hazard identification of pesticides as this is not designed to detect the particular changes that occur only during early (pre- and postnatal) life stages and models do not involve 'a second hit' that has been captured in experimental studies. The only available study that covers these critical stages is the Extended One-Generation Reproductive Toxicity Study (OECD TG 443); however, this is relatively recent and has not been applied to most of the pesticides currently marketed. Furthermore, the protocol was not designed to cover carcinogenic endpoints and the power of the study is probably not sufficient.

In short, there is a strong expectation that AOP development would provide biological plausibility for epidemiological observations, enabling to identify important etiological factors for complex human outcomes and to develop clinically useful biomarkers and thus support the improvement of hazard and risk assessment.

On the basis of the EFSA scientific opinion on the use of epidemiological studies for pesticide risk assessment (EFSA PPR Panel, 2017b), the conclusions involved the following important points: 1) AOP framework contributes to hazard identification and characterisation and it is useful in regulatory risk assessment to explore whether an AO (e.g., those identified in any OECD TG) is biologically plausible or not. However, chemical specific risk assessment benefits from MoA and/or IATA framework. 2) The prototype AOPs developed for PD and infant leukaemia supported the epidemiological findings indicating that pesticides interacting with specific MIEs and triggering downstream key events (KE) are indeed risk factors for PD and infant leukaemia. 3) The AOP framework is an appropriate tool to understand whether chemical hazards relevant to such human diseases can be explored and detected in standard

1 regulatory studies as well as to identify knowledge gaps in regulatory toxicology testing that
2 require to be addressed. Two papers stemming from the original project have already been
3 published in the publicly available scientific literature (Pelkonen et al 2017; Terron et al
4 2018).

5
6
7 A practical example to develop an AOP within the scope of the EFSA Scientific Opinion on
8 Investigation into experimental toxicological properties of plant protection products having a
9 potential link to PD and childhood leukaemia (EFSA PPR Panel, 2017a) is represented by the
10 inhibition of the mitochondrial complex I of dopaminergic nigrostriatal neurons leading to
11 parkinsonian motor deficits (Terron et al 2018). Because PD is a complex disease, the
12 description of its biological basis would probably need multiple AOPs with diverse MIEs,
13 KEs and AOs, which can even be shared among them or interact with one another. Details of
14 development of the specific AOP were outlined and the final AOP was submitted to OECD
15 (Bal-Price et al., 2018²) and has been adopted by the OECD). This specific AOP example
16 demonstrates that the AOP conceptual framework is a valid tool to provide a mechanistic
17 biological plausibility to the association found in epidemiological studies between exposure to
18 pesticides and PD and can inform IATA. Because individual pesticides may have several
19 pathways linked to this complex disease, development of AOP networks is a prerequisite for
20 the identification of mechanistically driven cumulative assessment groups for PD. In this
21 respect, another AOP was proposed (Viviani 2016)³. The foreseen testing strategy should
22 consider all KEs involved in the AOP followed by selection of the most predictive assays
23 (Bal-Price and Meek 2017; Bal-Price et al 2017). Furthermore, as recently exemplified, the
24 approach has been expanded for substance evaluation under the European Chemical
25 Regulation (REACH). A potential PD predisposition was suspected for the industrial
26 chemical and fungicide Zinc bis(dimethyldithiocarbamate) (known as Ziram in agriculture)
27 based on epidemiological and mechanistic data and on the approach reported by the EFSA
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

52
53 ² Bal-Price A, Leist M, Schildknecht S, Tschudi-Monnet F, Paini A, Terron A (2018), "Adverse Outcome Pathway
54 on Inhibition of the mitochondrial complex I of nigro-striatal neurons leading to parkinsonian motor deficits",
55 OECD Series on Adverse Outcome Pathways, No. 7, OECD Publishing, Paris, [https://doi.org/10.1787/b46c3c00-](https://doi.org/10.1787/b46c3c00-en)
56 [en](https://doi.org/10.1787/b46c3c00-en)., and Bal-Price A, Leist M, Schildknecht S, Tschudi-Monnet F, Paini A, Terron A. Inhibition of the
57 mitochondrial complex I of nigro-striatal neurons leads to parkinsonian motor deficits (AOP 3). Draft version
58 under review. <https://aopwiki.org/aops/3>

59 ³ Viviani B. Adverse Outcome Pathway: Redox-cycling of a chemical initiated by electrons released by the
60 mitochondrial respiratory chain leading to parkinsonian motor deficits. Toxicol Let 2016; 259S: S62
61
62
63
64
65

1 opinion (2017a). Specific investigation of the substantia nigra and dopaminergic neurons as
2 recommended in the EFSA opinion (2017a), was required.⁴
3

4 In the overall scenario of the applicability of the AOP framework for assessing causality of
5 observations in epidemiological studies, the following considerations should be accounted
6 for: distinction between AOP and chemical-specific MOA, evolution of Weight of Evidence
7 (WoE)/Confidence considerations in MOA/AOP analysis, and implications for assessment of
8 causality in epidemiological studies for regulatory application (Bhat et al 2017; Rhomberg et
9 al 2013). As mentioned above, AOPs are well suited to consideration of biological plausibility
10 for causation in epidemiological studies. Another crucial factor is the assessment of, and
11 confidence in, experimental support for AOPs. Besides, it is of importance to keep in mind
12 the distinction between confidence in a mechanistic pathway (AOPs) and replication of a
13 human effect in animal studies as support for biological plausibility underpinning causation in
14 epidemiological studies (Meek et al 2014). Obviously such an analysis has some implications
15 regarding planning, conduct and assessment of epidemiological studies for regulatory
16 application. These include the need for common “metrics” for exposure and outcome
17 assessment (whose elements should be precisely defined), analysis of confidence, inclusion of
18 appropriate elements into study designs and epidemiological training of researchers and
19 assessors. All these are required to facilitate purpose specific regulatory application.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

36 **Considerations and recommendations for future work**

37
38 On the basis of the above coverage of the EFSA project and scientific opinions as well as
39 additional considerations about strengths and limitations of pesticide epidemiological studies,
40 it is opportune to summarize the current situation as to what will be the most important future
41 needs and topics in research and development as follows:
42
43
44
45

46 ***Assessment of pesticide exposure***

47
48 Assessment of exposure is considered as the main issue when dealing with epidemiological
49 evidence on pesticides available so far, due to intrinsic difficulties in characterizing exposure
50 to individual active substances. Pesticide exposure can be modelled by using validated
51 questionnaires or job-exposure matrices, although biomonitoring can provide better metrics of
52
53
54
55
56
57
58

59 ⁴ https://echa.europa.eu/documents/10162/23715527/msc-57_minutes_en.pdf/11f11007-1814-48fc-5818-775f9e12e8ec.
60
61
62
63
64
65

1 exposure. “Exposome” approaches and molecular epidemiology open new possibilities for
2 research and advanced risk assessment bridging toxicology and epidemiology.

3
4 The exposome, that is, the totality of exposures received by an individual during life time
5 represents a challenging but promising new concept in the field and currently there are tools
6 available to measure exposome, e.g. biomarkers of the internal exposome (xenobiotics and
7 metabolites), or the use of -omic technologies (adductome, metabolome, transcriptome,
8 epigenome, proteome) (Vineis et al., 2017), although still only for research purposes.
9

10
11
12
13 Developments in the field of molecular epidemiology will improve exposure assessment,
14 document early changes in the toxicity pathway preceding disease, and identify subgroups in
15 the population with greater susceptibility to adverse outcomes. Thereby, the ability of
16 epidemiological studies to identify causal risk factors and elucidate mechanisms underlying
17 pathogenesis of diseases will increase. The implementation of molecular epidemiology tools,
18 especially connected with exposome, AOPs/MOAs and systems toxicology, will provide
19 additional possibilities for exposure assessment and health risk prediction.
20
21
22
23
24
25

26
27 It is important to consider what pesticide exposure actually means in the context of
28 epidemiology research and experimental research. In real life, long-term exposures are almost
29 always complex regarding both PPP and other chemical exposures (either simultaneously or
30 sequentially), whereas in experimental studies exposures are mostly to single pesticide active
31 substances and at high doses, which represents an unrealistic scenario. This dilemma creates
32 problems when risk assessments of combined exposure to multiple chemicals are performed.
33
34
35
36
37

38 ***The use of AOP as a scaffold to provide biological plausibility to epidemiological findings***

39
40
41 The AOP framework is a useful tool for risk assessment to explore whether an adverse
42 outcome is biologically plausible or not. By mechanistically substantiating apical endpoints or
43 outcomes, the AOP contributes to the inclusion of human data in hazard identification and
44 characterization steps in risk assessment. Thus, AOPs allow moving towards a mechanistic-
45 based risk assessment.
46
47
48
49

50
51 If strong epidemiological evidence is available, there is no need to use an AOP for going
52 ahead with risk assessment. However, even in this case, an AOP can still provide additional
53 support on a positive finding, especially on the identification of potential risk factors
54 (lifestyles, genetics, environmental chemicals, etc.) identified by the intermediate key events.
55
56 Where epidemiological studies of specific diseases (e.g. PD) would be time-consuming and
57 expensive, and often would identify individual pesticides or groups of pesticide, an AOP
58
59
60
61
62
63
64
65

would provide insight into their risk factors. This can be particularly useful for chronic human degenerative diseases where gene-environmental interactions strongly influence the risk, severity and progression of such diseases and where the ability of animal model of replicate the disease associated pathology is very limited..

In these cases, AOPs are built starting from adverse outcomes, thus matching a hazard profile of a specific exposure (chemical/stressor) interacting with a molecular initiating event (MIE) and triggering the linear chain of key events eventually leading to the AO. However, it seems unlikely that a single AOP can explain all endpoints of complex diseases.

In cases where modest or weak associations between adverse outcomes and exposures are found, AOPs would provide supportive evidence for the mechanistic biological plausibility or, contrary, negative evidence for the pathogenesis of a disease.

Quality assessment of human epidemiological studies

Quality in epidemiological studies represents an issue for individual studies, which covers from study design to study reporting, and for pooled evidence.

Key factors to determine whether epidemiology findings should be taken into account for a WoE assessment are addressed by assessing the risk of bias for observational epidemiological studies based on specific tools available (US-EPA, 2016; EFSA PPR Panel, 2017b). If this assessment is part of the evidence synthesis where epidemiological research is assessed and quantitatively summarised, it permits more accurate estimation of the magnitude of the effect related to pesticide exposure. This is an important point as pesticide risk assessment should not be based on results of epidemiological studies that do not meet well-defined data quality standards, because a high risk of bias challenges the internal validity of a study.

When a systematic review is conducted to synthesize evidence, assessment of methodological quality and risk of bias of the selected studies should be performed. Individual studies should be evaluated for possible selection bias, measurement error, sampling error, heterogeneity, study design, and reporting and presentation of results. In addition, meta-analysis allows for examining additional bias, such as small study effects, publication bias and excess significance bias.

Training issue as a necessary enabling factor

Experts from different disciplines are needed for the balanced integrated risk assessment of pesticides. There exists a consensus about expertise needed for the evaluation of

1 epidemiological evidence in risk assessment of pesticides. Ideally, epidemiologists trained in
2 (chemical) risk assessment are required as well as a permanent dialogue between
3 epidemiologists and toxicologists (and many other disciplinary experts at least occasionally if
4 needed, such as experts in exposure science).
5
6

7 Specific case studies on the use of epidemiological evidence for pesticide risk assessment
8 would be valuable especially for training purposes. US-EPA has some examples: Dicamba for
9 Tier I, 2,4-D and Permethrin for Tier II and Atrazine, Glyphosate and Chlorpyrifos for Tier
10 III.
11
12
13

14 ***Future Guidance from EFSA Scientific Committee***

15 Because this opinion piece presents EFSA experiences, it is proper to finalize with some
16 recommendations to EFSA: although there are also diverse views, it seems preferable that an
17 overarching Guidance should be drafted by the EFSA Scientific Committee regarding
18 chemicals in general, not only pesticides (this activity is foreseen).
19
20
21
22
23
24

25 Exposure assessment should be the prime consideration and investment to be made when
26 dealing with the EFSA guidance, because it is the most obvious gap in knowledge creating
27 uncertainty. However, for some chemicals, such as heavy metals, smoking, alcohol, or
28 organochlorine compounds, exposure can be properly characterized, although also these
29 exposures involve usually other simultaneously exposing multiple chemicals.
30
31
32
33
34

35 Vigilance observations, including medical data, are an under-sought source of information for
36 chemical risk assessment. A section in the future guidance for the use of such data should be
37 developed.
38
39
40
41

42 **Conclusions**

43 Risk assessment of pesticides is a complex task. Besides, regulatory toxicity tests may do not
44 fully address adverse effects observed in human epidemiological studies. On the other hand,
45 these may not be sensitive enough or focused to ever detect significant harmful effects.
46 Consequently, the following proposals would enhance a more human-relevant and hazard-
47 targeted risk assessment of pesticides:
48
49
50
51
52

- 53 • The use of the AOP conceptual framework to provide the mechanistic basis for a
54 biological plausible link between a MIE and an AO found in epidemiological studies.
- 55 • An initial framework for the evaluation and integration of epidemiological
56 observations in the pesticides risk assessment.
57
58
59
60
61
62
63
64
65

- Since the most obvious gap in the proposed approach is the complexity of performing an adequate exposure assessment, this should be overcome by implementing human biomonitoring, –omic technologies, or exposome analysis, which takes a holistic approach by combining data from multiple sources.
- A guidance to facilitate the risk assessment process of chemicals in general by using a multidisciplinary approach.

References

- Agricultural Health Study (AHS). <https://aghealth.nih.gov/>
- Andreotti G, Koutros S, Hofmann JN, Sandler DP, Lubin JH, Lynch CF, Lerro CC, De Roos AJ, Parks CG, Alavanja MC, Silverman DT, Beane Freeman LE (2018) Glyphosate Use and Cancer Incidence in the Agricultural Health Study. *J Natl Cancer Inst* 110:509-516.
- Bal-Price A, Meek MEB (2017) Adverse outcome pathways: Application to enhance mechanistic understanding of neurotoxicity. *Pharmacol Ther* 179:84-95. doi: 10.1016/j.pharmthera.2017.05.006.
- Bal-Price A, Lein PJ, Keil KP, Sethi S, Shafer T, Barenys M, Fritsche E, Sachana M, Meek ME (2017) Developing and applying the adverse outcome pathway concept for understanding and predicting neurotoxicity. *Neurotoxicology* 59:240-255. doi: 10.1016/j.neuro.2016.05.010.
- Bhat VS, Meek MEB, Valcke M, English C, Boobis A, Brown R (2017) Evolution of chemical-specific adjustment factors (CSAF) based on recent international experience; increasing utility and facilitating regulatory acceptance. *Crit Rev Toxicol* 47(9):729-749. doi: 10.1080/10408444.2017.1303818.
- Choi J, Polcher A, Joas A (2016) 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/publication
- Delrue N, Sachana M, Sakuratani Y, Gourmelon A, Leinala E, Diderich R (2016) The adverse outcome pathway concept: A basis for developing regulatory decision-making tools. *Altern Lab Anim* 44(5):417-429.
- Edwards SW, Tan YM, Villeneuve DL, Meek ME, McQueen CA (2016) Adverse Outcome Pathways-Organizing Toxicological Information to Improve Decision Making. *J Pharmacol Exp Ther* 356(1):170-81. doi: 10.1124/jpet.115.228239.
- EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), Ockleford C, Adriaanse P, Berny P, Brock T, Duquesne S, Grilli S, Hernandez-Jerez AF, Bennekou SH, Klein M, Kuhl T, Laskowski R, Machera K, Pelkonen O, Pieper S, Smith R, Stemmer M, Sundh I, Teodorovic I, Tiktak A, Topping CJ, Wolterink G, Angeli K, Fritsche E, Hernandez-Jerez AF, Leist M, Mantovani A, Menendez P, Pelkonen O, Price A, Viviani B, Chiusolo A, Ruffo F, Terron A and Bennekou SH (2017a) Scientific Opinion on the

investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia. EFSA Journal 2017a; 15(3):4691, 325 pp. doi:10.2903/j.efsa.2017.4691

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), Colin

Ockleford, Paulien Adriaanse, Philippe Berny, Theodorus Brock, Sabine Duquesne, Sandro Grilli, Susanne Hougaard, Michael Klein, Thomas Kuhl, Ryszard Laskowski, Kyriaki Machera, Olavi Pelkonen, Silvia Pieper, Rob Smith, Michael Stemmer, Ingvar Sundh, Ivana Teodorovic, Aaldrik Tiktak, Chris J. Topping, Gerrit Wolterink, Matteo Bottai, Thohallur Halldorsson, Paul Hamey, Marie-Odile Rambourg, Ioanna Tzoulaki, Daniele Court Marques, Federica Crivellente, Hubert Deluyker and Antonio F.

Hernandez-Jerez (2017b) Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report "Literature review of epidemiological studies linking exposure to pesticides and health effects". EFSA Journal 2017b; 15(10):5007, 101 pp. <https://doi.org/10.2903/j.efsa.2017.5007>

Fritsche E, Crofton KM, Hernandez AF, Hougaard Bennekou S, Leist M, Bal-Price A, Reaves E, Wilks MF, Terron A, Solecki R, Sachana M, Gourmelon A (2017) OECD/EFSA workshop on developmental neurotoxicity (DNT): The use of non-animal test methods for regulatory purposes. ALTEX 34(2):311-315. doi: 10.14573/altex.1701171.

Goodman M, Naiman DQ, LaKind JS (2018) Systematic review of the literature on triclosan and health outcomes in humans. Crit Rev Toxicol 48(1):1-51. doi: 10.1080/10408444.2017.1350138.

Hernandez AF, Tsatsakis AM (2017) Human exposure to chemical mixtures: Challenges for the integration of toxicology with epidemiology data in risk assessment. Food Chem Toxicol 103:188-193. doi: 10.1016/j.fct.2017.03.012.

Hernandez AF, Menendez P (2016) Linking Pesticide Exposure with Pediatric Leukemia: Potential Underlying Mechanisms. Int J Mol Sci 17(4):461. doi: 10.3390/ijms17040461.

LaKind JS, Sobus JR, Goodman M, Barr DB, Först P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teeguarden J, Tornero-Velez R, Weisel CP (2014) A proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument. Environ Int 73:195-207. doi: 10.1016/j.envint.2014.07.011.

LaKind JA, Idri F, Naiman DQ, Verner MA (2019) Biomonitoring and Nonpersistent Chemicals—Understanding and Addressing Variability and Exposure Misclassification. Current Environmental Health Reports <https://doi.org/10.1007/s40572-019-0227-2>

- Meek ME, Palermo CM, Bachman AN, North CM, Jeffrey Lewis R (2014) Mode of action human relevance (species concordance) framework: Evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. *J Appl Toxicol* 34(6):595-606. doi: 10.1002/jat.2984.
- Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA, PRISMA-P Group (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015 Jan 1;4:1. doi:10.1186/2046-4053-4-1.
- Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I (2013) Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication 2013:EN-497, 159 pp.
- Pelkonen O, Terron A, Hernandez AF, Menendez P, Bennekou SH, EFSA WG EPI1 Members (2017) Chemical exposure and infant leukaemia: development of an adverse outcome pathway (AOP) for aetiology and risk assessment research. *Arch Toxicol* 91(8): 2763-2780. doi: 10.1007/s00204-017-1986-x
- Rhomberg LR, Goodman JE, Bailey LA, Prueitt RL, Beck NB, Bevan C, Honeycutt M, Kaminski NE, Paoli G, Pottenger LH, Scherer RW, Wise KC, Becker RA (2013) A survey of frameworks for best practices in weight-of-evidence analyses. *Crit Rev Toxicol* 43(9):753-84. doi: 10.3109/10408444.2013.832727.
- Sakuratani Y, Horie M, Leinala E (2018) Integrated Approaches to Testing and Assessment: OECD Activities on the Development and Use of Adverse Outcome Pathways and Case Studies. *Basic Clin Pharmacol Toxicol* 123 Suppl5:20-28. doi: 10.1111/bcpt.12955.
- SAPEA, Science Advice for Policy by European Academies (2018) Improving authorisation processes for plant protection products in Europe: a scientific perspective on the potential risks to human health. Berlin: SAPEA. <https://doi.org/10.26356/plantprotectionproducts>
- Scientific Advice Mechanism (SAM) (2018) EU authorisation processes of Plant Protection Products from a scientific point of view. ISBN 978-92-79-67735-9 doi:10.2777/238919 KI-04-17-354-EN-N.
- Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA; PRISMA-P Group (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015 Jan 2;350:g7647. doi: 10.1136/bmj.g7647.

Terron A, Bal-Price A, Pains A, Monnet-Tschudi F, Bennekou SH; EFSA WG EPI1
Members, Leist M, Schildknecht S (2018) An adverse outcome pathway for parkinsonian
motor deficits associated with mitochondrial complex I inhibition. Arch Toxicol 92(1):41-
82. doi: 10.1007/s00204-017-2133-4.

US-EPA (United States Environmental Protection Agency) (2016) Office of Pesticide
Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk
Assessments for Pesticides December 28, 2016 [https://www3.epa.gov/pesticides/EPA-HQ-
OPP-2008-0316-DRAFT-0075.pdf](https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf)

Vineis P, Chadeau-Hyam M, Gmuender H, Gulliver J, Herceg Z, Kleijnans J, Kogevinas M,
Kyrtopoulos S, Nieuwenhuijsen M, Phillips DH, Probst-Hensch N, Scalbert A, Vermeulen
R, Wild CP (2017) EXPOsOMICS Consortium. The exposome in practice: Design of the
EXPOsOMICS project. Int J Hyg Environ Health 220:142-151.

Internet sources used in the article

Adverse outcome pathway knowledge base (AOP-KB): <http://aopkb.org/>

AOP-wiki: <https://aopwiki.org/>

Legend for Figure 1. The project landscape of the EFSA scientific opinions on Parkinson's disease and childhood leukaemia and on integration of epidemiology and experimental research. Time frame (upper part), epidemiological studies (middle part) and AOP framework (lower part) schematically presented.

Legend for Figure 2. Use of epidemiological evidence for pesticide risk assessment: strengths and limitations