Regulatory Toxicology and Pharmacology 83 (2017) 89–99

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Approaches for grouping of pesticides into cumulative assessment groups for risk assessment of pesticide residues in food

Thomas Colnot [a], Wolfgang Dekant [b, *]

[a] CiS Toxicology, Osorno, Chile
[b] Department of Toxicology, University of Würzburg, Versbacher Strasse 9, 97078, Würzburg, Germany

ARTICLE INFO

Article history:
Received 14 October 2016
Received in revised form
6 December 2016
Accepted 7 December 2016
Available online 10 December 2016

Keywords:
Cumulative risk assessment
Pesticides
Organophosphates
Thyroid
Margin of exposure
Human relevance
Nervous system
Cumulative assessment groups (CAGs)

ABSTRACT

The European Food Safety Authority (EFSA) is developing approaches to cumulative risk assessment of pesticides by assigning individual pesticides to cumulative assessment groups (CAGs). For assignment to CAGs, EFSA recommended to rely on adverse effects on the specific target system. Contractors to EFSA have proposed to allocate individual pesticides into CAGs relying on NOAELs for effects on target organs. This manuscript evaluates the assignments by applying EFSAs criteria to the CAGs "Toxicity to the nervous system" and "Toxicity to the thyroid hormone system (gland or hormones)". Assignment to the CAG "Toxicity to the nervous system" based, for example, on neurochemical effects like choline esterase inhibition is well supported, whereas assignment to the CAG "Toxicity to the thyroid hormone system (gland or hormones)" has been based in the examined case studies on non-reproducible effects seen in single studies or on observations that are not adverse. Therefore, a more detailed effects evaluation is required to assign a pesticide to a CAG for a target organ where many confounders regarding effects are present. Relative potency factors in cumulative risk assessment should be based on benchmark doses from studies in one species with identical study design and human relevance of effects on specific target organs should be analyzed to define minimal margins of exposure.

1. Introduction

EFSA has recommended the concept of dose addition for risk characterization of mixtures of pesticides even for pesticides with a dissimilar mode of action if they produce a common and adverse outcome on a target organ/system (EFSA-PPR-Panel, 2013b, 2014). This is a conservative and pragmatic default approach since mode of action data regarding induction of adverse effects are often limited (EC-SCHER, 2012). Therefore, individual pesticides inducing a common adverse outcome in the same target organ/system should be grouped into „common assessment groups" (CAGs) for cumulative human health risk characterization. The tiered grouping approach consists of several levels, level 1 is a common target organ and level 2 specifically describes the phenomenological effect (EFSA-PPR-Panel, 2013b, 2014). At levels 3 and 4, additional mode and/or mechanism of action information could be integrated. However, such information is not available for the majority of pesticides. Thus, grouping into CAGs is currently based on phenomenological effects at level 2.

Cumulative risk assessment requires both identification of relevant adverse effects on the target organ/system and a comparative assessment of potency of the individual compounds in a CAG. Identification of relevant toxic effects requires a thorough hazard assessment considering information from all toxicity studies available and its consistency. Relative potency may be expressed by No-Observed-Adverse-Effect-Levels" (NOAELs) or benchmark doses (BMDs) for the common and adverse outcome. The relative potency of individual members in a CAG, in combination with exposure characteristics, may have significant consequences for tolerable maximum residue levels for individual pesticides in food. Therefore, assignment of pesticides to CAGs and assessment of relative potency have to be based on the best available science using the most appropriate methodology (Kienzler et al., 2016).

Regarding inclusion of a pesticide into a target organ/system specific CAG, EFSA has developed a methodology to identify effects of an individual pesticide on target organs/systems. The methodology proposes a stepwise approach with a major focus on identification and characterization of the specific effect. EFSA apparently

* Corresponding author.
E-mail address: dekant@toxi.uni-wuerzburg.de (W. Dekant).

http://dx.doi.org/10.1016/j.yrtph.2016.12.004

relies on systemic "adverse effects" as defined by IPCS (WHO/IPCS, 2004) since "local effects", "non-adverse effects", and "non-specific effects" should be excluded. In addition, only "unambiguous effects" (EFSA-PPR-Panel, 2013a) interpreted here as "clear adverse effects of sufficient magnitude and biological relevance as a consequence of administration of a specific chemical" should be considered. Effects without human relevance should also not be considered. This is a reasonable approach following well-established procedures (EFSA-PPR-Panel, 2014; Dekant and Bridges, 2016).

Contractors to EFSA have already proposed to include a number of active pesticides into specific CAGs (level 2) with a focus on the nervous system and the thyroid (supplementary material, Table 1s). The nervous system CAG was further subdivided into CAGs for acute effects on motor, sensory, and autonomic divisions of the nervous system, and into neurochemical endpoints. Pesticides with effects on the thyroid were allocated to CAGs for effects either on C-cells and the calcitonin system or to a group affecting thyroid hormones (T3/T4) and the thyroid follicular cells. The CAG-group "thyroid follicular cell hypertrophy/hyperplasia and/or increased relative thyroid weight" has the largest number of pesticides included (EFSA-PPR-Panel, 2013b).

Effects to be used as a basis for assignment to a CAG and organ-specific NOAELs and LOAELs were derived by contractors to EFSA. In this context, the "*selection of NOAELs and LOAELs was performed, as requested by EFSA, without any interpretation of whether an effect is to be considered as adverse or not adverse*" implying that any effect reported was used. Apparently, there was no consideration of study quality, dose-response, or consistency of effects over studies. This procedure is inconsistent with the practice of toxicological risk characterization and with the basic approach outlined by EFSA (EFSA-PPR-Panel, 2014). Assessment of adversity and unambiguity of an effect requires a detailed evaluation of the database and cannot be limited to giving NOAELs/LOAELs.

Apparently, this very wide definition served as an initial screening only and requires further consideration along the lines outlined in the guidance documents. Therefore, this manuscript evaluates the results of the grouping of pesticides into CAGs with focus on the level 2 CAGs "thyroid follicular cell/T3/4 system" and "neurochemical effects" regarding support for inclusion of a pesticide into the respective CAG using a selected set of pesticides (cyhalofop-butyl, dithianon, ametoctradin, amidosulfuron) (EFSA-PPR-Panel, 2014; RIVM-ICPS-ANSES, 2016). Datasets for these pesticides were evaluated by applying the criteria of weight-of-evidence and adversity based on the European Peer Review conclusions, the Draft Assessment Reports (DARs) and original study reports. In addition, the manuscript proposes approaches to derive robust potency factors based on adverse effects to serve as a basis for the cumulative assessment. Our approach is in line with EFSAs SC recommendation for risk characterization that focus on "unambiguous" and "adverse effects" (EFSA-PPR-Panel, 2013a).

2. Methodology to select appropriate studies as a basis for assignment of pesticides to the level 2 CAGs "neurochemical group" and "thyroid follicular cell/T3/T4 system"

2.1. General criteria for study evaluation

A solid assessment of the hazardous properties of a chemical requires both a quality assessment of the respective study to judge the reliability of the study outcome and an evaluation of the toxic effects reported to judge their adversity and consistency regarding different levels of the biological response from biomarkers to functional and structural changes. In many cases, quality assessment of a toxicity study is based on an application of the Klimisch scale or other rather superficial approaches (Klimisch et al., 1997; Lutter et al., 2015). The major limitation of the Klimisch scale is that "reliability without restrictions" is only allocated to studies performed following OECD study guidelines with full study reports containing raw data. Other studies including those published in the peer-reviewed scientific literature are termed "reliable with restrictions". However, the restrictions regarding reliability are not explicitly defined and a systematic approach to determine reliability is not included. Therefore, the criteria of the Klimisch scale focus more on the quality of reporting and not the quality of the actual work. For this reason, a more detailed quality assessment is required (Dekant and Bridges, 2016). Quality assessment needs to include the extent of characterization of the test chemical, its stability in the application medium, and potential presence of contaminants. Evaluation of the study design needs to consider the species selected, the number of animals/dose group and the number and spacing of dose levels, the appropriateness of the dose-range tested, the inclusion of appropriate controls, and the relevance of the route and timing of administration. Other important issues are the reliability of the applied methodology for analytical measurements (such as thyroid hormones or cholinesterase activity) and potential issues with sample preparation for histopathology. Finally, the appropriateness of procedures for statistical evaluation of incidence data needs to be evaluated and, in case of equivocal effects, compared to historical control data for the selected endpoints.

Effects assessment also requires a detailed analysis of the reported changes. As EFSA apparently proposes to use "adverse effects" as a basis to include specific chemicals into CAGs, adversity of all changes reported needs to be determined. A critical issue in the determination of adversity consists in the question if changes are really adverse and biologically relevant, represent adaptive or physiological changes, or are random events not related to treatment. Adverse effects are defined by IPCS as those "*that result in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences*" (WHO/IPCS, 2004), while non-adverse or adaptive effects are those "*biochemical, morphological, or physiological changes that do not affect the general well-being, growth, development or life span*" (Lewis et al., 2002).

To qualify as adverse, effects reported in a study usually are required to exhibit a dose-response and be consistent with the other changes regarding hypothesis of disease development, e.g. an adverse outcome pathway (Carmichael et al., 2011; Simon et al., 2014; Sturla et al., 2014). In this analysis, a change in a parameter is considered adverse if it shows a dose response with higher incidences/intensity at higher doses. Moreover, when biomarkers such as changes in enzyme activity or hormone levels are used to derive a point-of-departure (POD) for risk characterization, the change in the biomarker is considered as biologically significant only when adverse effects were observed at higher doses or after longer exposures. Assessment of the relevance of changes in biomarkers and adverse effects on a target organ also requires to consider the possibility that reported changes are secondary to other effects induced by the treatment. For example, when there are clear indications of liver changes such as increased liver weight due to induction of biotransformation enzymes, deriving a potency factor regarding thyroid changes at higher doses is inappropriate since such changes are a consequence of the modified disposition of thyroid hormones that only occurs after administration of doses that result in significant induction of xenobiotic metabolizing enzymes. In case of conflicting information, a detailed weight-of-evidence analysis is required to decide if a biomarker changes should be used as a basis of a POD (Lamb et al., 2015; Lutter et al., 2015).