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Contemporary Review

A comprehensive view on mechanistic approaches for cancer risk assessment of non-genotoxic agrochemicals

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ABSTRACT

Abbreviations: ADME, (absorption, distribution, metabolism and excretion); Adverse Outcome Pathways, (AOPs); Classification and Labelling, (CLP); European Chemicals Agency, (ECHA); European Commission, (EC); European Food Safety Authority, (EFSA); European Partnership on Alternative Approaches to Animal Testing, (EPAA); Fungicide, Insecticide, and Rodenticide Act, (FIFRA); hypothalamic-pituitary-thyroid, (HPT); hypothalamic-pituitary-gonadal, (HPG); integrated approach to the testing and assessment, (IATA); International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, (ICH); Key events, (KEs); modes of action, (MOAs); Negative for Endocrine, Genotoxicity, and Chronic Study Associated Histopathologic Risk Factors for Carcinogenicity, ("NegCarc"); non-genotoxic carcinogens, (NGTXC); non-governmental organizations, (NGOs); Organisation for Economic Co-operation and Development, (OECD); PETA International Science Consortium Ltd, (PETA-ISC); QSAR, (quantitative structure–activity relationships); reduction, replacement and refinement of animals; (3Rs), Registration, Evaluation, Authorisation and Restriction of Chemicals; (REACH), Rethinking Carcinogenicity Assessment for Agrochemicals Project; (ReCAAP) Toxic Substances Control Act, (TSCA); US Environmental Protection Agency, (US EPA); Working Group of National Coordinators for the Test Guidelines Programme, (WNT); World Health Organization / International Programme on Chemical Safety, (WHO/IPCS).

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Currently the only methods for non-genotoxic carcinogenic hazard assessment accepted by most regulatory authorities are lifetime carcinogenicity studies. However, these involve the use of large numbers of animals and the relevance of their predictive power and results has been scientifically challenged. With increased availability of innovative test methods and enhanced understanding of carcinogenic processes, it is believed that tumour formation can now be better predicted using mechanistic information. A workshop organised by the European Partnership on Alternative Approaches to Animal Testing brought together experts to discuss an alternative, mechanism-based approach for cancer risk assessment of agrochemicals. Data from a toolbox of test methods for detecting modes of action (MOAs) underlying non-genotoxic carcinogenicity are combined with information from subchronic toxicity studies in a weight-of-evidence approach to identify carcinogenic potential of a test substance. The workshop included interactive sessions to discuss the approach using case studies. These showed that fine-tuning is needed, to build confidence in the proposed approach, to ensure scientific correctness, and to address different regulatory needs. This novel approach was considered realistic, and its regulatory acceptance and implementation can be facilitated in the coming years through continued dialogue between all stakeholders and building confidence in alternative approaches.

1. Introduction

To ensure protection of human health, the majority of chemical substances are required by regulations to undergo hazard and risk assessments before being marketed (applicable laws include, but are not limited to, *e.g.* the European Commission regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH (EC, 2008)), the European Commission regulation on placing plant protection products on the market (EC, 2009; EC, 2013), the United States Toxic Substances Control Act (TSCA) (US EPA, 2016), and the United States FIFRA (Fungicide, Insecticide, and Rodenticide Act) pesticide registration data requirements (Jaeger, 1984). Depending on the use of the chemical, one of the hazards for which data is required is carcinogenicity. For agrochemicals, the current standard approach for assessing human carcinogenic potential is to conduct a series of assays for genotoxicity, and two rodent carcinogenicity studies. Based on the assumption that genetic damage results in human-relevant tumour formation, genotoxic carcinogens can be identified using robust and predictive short-term *in vitro* and *in vivo* assays (EFSA, 2011; Luijten et al., 2016; US EPA, 1986). A number of chemicals remain that induce cancer *in vivo,* but show no evidence of genotoxicity. These are termed non-genotoxic carcinogens (NGTXC). Identification of NGTXC is more difficult compared to genotoxic substances and has traditionally relied on rodent carcinogenicity studies (Hernandez et al., 2009). However, in recent years, there has been increasing momentum to find alternatives to the carcinogenicity assay, since there is considerable scientific doubt regarding the reliability of the model (Ames and Gold, 1990; Smith and Perfetti, 2018) due to a high number of false positive results arising from long-term exposure of animals to high doses of the test substance (Anisimov et al., 2005; Haseman et al., 1998; Jacobson-Kram et al., 2004), and a low reproducibility (Gottmann et al., 2001) making relevance to human risk assessment questionable (Annys et al., 2014; Cohen et al., 2019; Goodman, 2018; Paparella et al., 2017). In addition, testing on rodents is expensive, time-consuming and uses a large number of animals. Since the introduction of the long-term carcinogenicity study in the early 1960s, there is a much better understanding of the mechanisms involved in the process of both rodent and human carcinogenicity and a huge advance in the development of methods to measure internal and exposure levels of chemicals and their effects on targeted organs. This has led to changes in how safety assessment for carcinogenicity could be evaluated in the future using more fit-for-purpose assays and fewer animals to generate more relevant data. Therefore, the European Partnership for Alternative Approaches to animal testing (EPAA), a collaboration between the European Commission, European trade associations and companies from various industry sectors, funded a project to develop and evaluate the applicability of an alternative, mechanism-based approach for predicting the carcinogenic potential of agrochemicals. To discuss the proposed approach with various stakeholders, a workshop entitled "Mechanism-based approach to cancer risk assessment incorporating 3Rs principles" was organised by the EPAA in Brussels on 12–13 June 2019. Approximately 30 experts participated in the event, including scientists working in regulatory and public health agencies, public administration, the chemical, agrochemical, pharmaceutical industry and non-governmental organizations (NGOs). The present manuscript provides some background and describes the outcomes of the discussions held at the workshop.

2. Background to the EPAA 2019 workshop: sector-specific regulations and practices in non-genotoxic carcinogenicity assessment

In 2011, the EPAA reviewed testing requirements for regulatory toxicology in different sectors to evaluate cross-sector alignment on best practices and 3Rs (reduction, replacement and refinement of animals) opportunities. As a result, carcinogenicity was identified as an area where there were opportunities to harmonise approaches and advance the 3Rs, due to differences in regulatory requirements across sectors and regions, as well as the length of the assay and the high use of animals. Consequently, the EPAA organised a workshop in 2013, where participants from a wide range of sectors met to discuss areas in which carcinogenicity testing methods could be aligned and contribute to reducing the number of animals used for hazard evaluation and risk assessment (Annys et al., 2014). Results from an EPAA survey presented at the workshop indicated that while all EU regulatory agencies require carcinogenicity assessment to support product marketing, the extent of testing and the types of studies differed widely across sectors (an overview of regulatory requirements in different sectors was presented in the paper of Annys et al. (2014)). The different requirements are based on factors such as anticipated level of exposure, specific concerns (*e.g.* perception of risks), available information gathered by other toxicity tests, and final use scenarios of the compound (*e.g.* short-versus long--term use). Regulatory approaches in the different sectors had varying degrees of flexibility in requirements for carcinogenicity testing. For example, in the EU the industrial chemicals sector uses an integrated approach based on manufacturing tonnage (an indication of potential exposure) and the results of genotoxicity testing, whereas the veterinary sector places more emphasis on genotoxicity and structural similarities to known (groups of) carcinogens. The regulatory requirements for agrochemical and biocidal active substances include two carcinogenicity studies conducted in rats and mice; typically, these tests are conducted on non-genotoxic compounds, since genotoxic substances are not further developed to market. Despite these differences, the 2013 workshop participants agreed that there are opportunities to harmonise testing requirements that would provide 3Rs benefits through changes to the design and conduct of carcinogenicity studies.

3. The EPAA project on carcinogenicity of pharmaceuticals

An alternative approach to assessing carcinogenic potential was published by a consortium of pharmaceutical companies: the so-called

"NegCarc" (Negative for Endocrine, Genotoxicity, and Chronic Study Associated Histopathologic Risk Factors for Carcinogenicity) approach (Sistare et al., 2011). This approach, developed based on data from 182 pharmaceuticals, relies on histopathological information from 6-month subchronic repeated dose toxicity studies, in addition to information regarding hormonal perturbation and genetic toxicity. The authors suggested to use three criteria, *i.e.* absence of preneoplastic lesions, together with a lack of evidence of hormonal imbalance and genotoxic potential, to predict a lack of tumour formation in a carcinogenicity study. Despite a reasonable negative predictivity (*i.e.* 82%), there were some concerns hampering regulatory adoption of the approach, arising - at least in part - from the definition of the three criteria and how they are applied (Bourcier et al., 2015). Further inspection of the dataset used for developing the NegCarc approach led to the hypothesis that predictions of carcinogenic potential could be improved by also taking into account pharmacologic properties (ICH, 2013). This hypothesis was evaluated by van der Laan and colleagues and proven to be valid in two previous projects, one of which was an EPAA project (van der Laan et al., 2016a, b). They confirmed the association of negative findings for the three criteria with negative carcinogenicity. Moreover, they showed that negative and positive predictivities increased to 92 and 98%, respectively, when pharmacological properties were also taken into account (van der Laan et al., 2016b). The International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is evaluating this weight-of-evidence approach in a prospective evaluation period, to analyse whether this might be sufficient for establishing the carcinogenic potential of pharmaceuticals without conducting life-time rodent assays (ICH, 2013; van der Laan et al., 2019).

4. The EPAA project on carcinogenicity of agrochemicals

The current EPAA project on NGTXC builds on the previous experience with the approach for pharmaceutical products and the evaluation of testing requirements in different sectors. The overall goal of this project is to provide evidence that data from subchronic repeated dose toxicity studies can be leveraged to predict human relevant carcinogenic potential of agrochemicals with reduced or no need for a carcinogenicity study. The focus is on agrochemicals, which, according to current regulatory requirements, involve the mandatory conduct of long-term carcinogenicity studies in two species (OECD Test Guideline 451 (OECD, 2018) as part of their risk assessment.

In a first step, a database of reports for over 400 agrochemicals evaluated by the US Environmental Protection Agency (US EPA) and the European Chemicals Agency (ECHA; Classification & Labelling inventory) was used to identify and categorise tumours induced by chronic administration of the chemical. For some of these agrochemicals, information was incomplete or insufficient. The remaining 352 chemicals were categorised into (i) genotoxic carcinogens (25); (ii) NGTXC (170); and (iii) non-carcinogenic chemicals (157). Of the 170 NGTXC with sufficient quality data, information on tumours was listed, including sites, sex, and species. A more detailed description of these findings has been published separately (Heusinkveld et al., 2020). Tumour sites were mainly in the liver and thyroid gland but others were evident in a wide range of tissues, including endocrine tissues and haematopoietic/lymphoid tissues. Where available, data related to mechanisms underlying tumour formation were collected from reports and/or from literature or, alternatively, they were deduced from the histopathological changes observed. The mechanistic information was then structured according to the concept of Adverse Outcome Pathways (AOPs), to serve as guidance for the selection of appropriate, preferably non-animal, assays. The goal is to develop a mechanism-based approach to cancer risk assessment, which combines (1) targeted information from subchronic toxicity studies (the NegCarc approach) with (2) a toolbox of test methods for detecting MOAs underlying non-genotoxic carcinogenicity. In time, this approach could potentially allow waivers for the long-term carcinogenicity study in the risk assessment of agrochemicals.

5. Workshop on feasibility of mechanism-based approach for predicting carcinogenic

5.1. Potential

To ensure engagement of various stakeholders and to collect valuable feedback, a workshop was held to discuss the mechanism-based approach for predicting the carcinogenic potential of agrochemicals. To illustrate what the mechanism-based approach may look like in practice, four "real-life" case studies from the database were developed and anonymised. These case studies were selected based on good availability of data; and they were merely used as examples to facilitate discussion of the proposed mechanism-based approach. They were presented to the participants. Each case study focused on a specific MOA and comprised data from *in vitro* and short-term *in vivo* studies for a specific agrochemical, while information on absence or presence of tumour formation was not included. For the purpose of this discussion, the focus was on information known about MOAs for tumours observed in rodent studies. At this stage, human relevance was not taken into account, however some participants noted that for these particular case studies, the MOAs do not have high human relevance. The following MOAs were studied in case studies 1–4:

1. Activation of CAR/PXR leading to induction of hepatocellular proliferation, hypertrophy/hyperplasia and eventually hepatocellular tumours (Elcombe et al., 2014; Peffer et al., 2018);
2. Activation of the hypothalamic-pituitary-thyroid (HPT) axis: induction of liver enzymes that metabolize thyroid hormones, leading to increased thyroid stimulating hormone production by the pituitary, followed by thyroid hypertrophy, hyperplasia and eventually thyroid tumours (Marty et al., 2015);
3. Activation of the HPT and hypothalamic-pituitary-gonadal HPG-axis, leading to pituitary hypertrophy and eventually pituitary tumours;
4. Activation of the HPG axis: induction of liver enzymes that metabolize testosterone, leading to increased production of luteinizing hormone by the pituitary, followed by Leydig cell hypertrophy, hyperplasia and eventually testis tumours (Marty et al., 2015; Papineni et al., 2015).

The specific aims of the case studies were to facilitate discussions as to whether data generated under a mechanism-based approach would provide sufficient evidence (both as dose and temporal concordance) to determine whether the MOA under study is perturbed by the particular agrochemical and to conclude whether it is carcinogenic in experimental animals. The discussions highlighted key elements for consideration, as described below.

6. Key elements for consideration in a mechanism-based approach

6.1. Regulatory needs

Above all, the new approach needs to fulfil all global regulatory requirements, namely risk assessment and Classification and Labelling (CLP) purposes (*e.g.* CLP; EC, 2008). Therefore, the approach needs to satisfy hazard identification but also provide the necessary information to meet the different regulatory needs.

6.2. Exposure

As a fundamental pillar of risk assessment, exposure considerations are necessary. The mechanistic approach should therefore include the possibility to use the data for a scientifically and regulatory appropriate risk assessment. It is expected that early key events (KEs) in the MOA

framework occur at lower concentrations of a stressor than *in vivo* neoplasms and that, therefore, an appropriate substance-specific toxicokinetic modelling would be provided for risk assessment.

6.3. Toxicokinetic properties

Understanding the ADME (absorption, distribution, metabolism and excretion) profile and toxicokinetic properties of a test substance will provide better understanding of relevance to human exposure, *i.e.* to inform the levels and duration of systemic exposure and the primary organs that will be exposed. Hence, the fate of a chemical after exposure should be investigated, including insight into potential species differences in metabolism, metabolism kinetics and potential saturation and/or switching of pathways across different doses. Therefore, appropriate assays to assess these parameters should be included in the toolbox (see below). This means that for some MOAs, a 7-day repeated dose toxicity study may not be sufficient to assess all toxicokinetic aspects; longer-term studies may be needed to achieve a steady state.

6.4. Relevant MOAs

The approach proposed is MOA-driven, with MOA considered upfront, rather than deducing the mechanisms from available data later after (new) studies have been conducted (obtained from *e.g.* standard toxicity studies). This requires an overview and identification of MOAs relevant for chemical-induced carcinogenesis, which share increased cell proliferation as a common event (Cohen and Ellwein, 1991). Whether both animal- and human-relevant pathways should be included remains to be discussed. One may argue to include only human-relevant pathways for human health risk assessment; on the other hand, broad knowledge of a MOA and its associated KEs has proven valuable for hazard assessment and may facilitate the transition towards an alternative approach. Thus far, the analysis conducted in this EPAA project has resulted in the identification of nine MOAs or MOA networks (Heusinkveld et al., 2020). A selection of these MOA networks was used to foster the discussion on the alternative approach. Not all MOAs are equally well understood nor are their interactions, and there might be additional, possibly unknown, MOAs that should be included in the approach. Relevant MOAs to be added may also include MOAs involved in human cancers for which the animal models commonly used for toxicity testing are poor predictors. Therefore, the relevant MOAs to be included in the approach should not only be based on data from animal studies, but also on clinical and epidemiological cancer data. It is also possible that a compound might act via mixed MOAs, and the different MOAs may be activated at different dose levels. Consequently, the establishment of the mechanism-based approach as well as building confidence with end-users are critical steps to ensure acceptance and implementation of the proposed approach. The latter may be facilitated by having a transition period in which, analogous to the pharmaceutical sector, a waiver based on a mechanism-informed weight-of-evidence is used and information identifying the MOA is included in a dossier.

6.5. Human relevance

The relevance of the identified MOAs for human health risk assessment needs to be evaluated. For data-rich MOAs, such as CAR-mediated liver tumours (Peffer et al., 2018), this might be feasible using the framework developed by the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) (Boobis et al., 2006; Meek et al., 2003, 2014). For other MOAs, additional research may be required, especially since differences in species relevance are in most cases quantitative rather than qualitative in nature.

6.6. Quantitative understanding of key event relationships

Initially, the mechanism-based approach aims at a qualitative prediction, *i.e.* presence or absence of carcinogenic potential. Such an approach should combine a high negative and a high positive predictive value to safeguard human health. In order to be fully applicable for risk assessment purposes, the approach should include quantitative understanding of the dose- and time-relationships between KEs in each of the relevant MOAs. Such understanding will allow for derivation of a point-of-departure for cancer risk assessment, by extrapolating from early to late KEs. It can also inform on the timing of when to measure early KEs of MOAs involved in carcinogenesis. For example, mitogen-induced cell proliferation resulting from CAR/PXR activation (case study #1) is an event that occurs within days rather than weeks and should thus be measured in a 3- or 7-day repeated dose toxicity study instead of a 90-day toxicity study. Quantification and understanding of the dose and time concordance of the MOA is therefore a critical step in defining the new point of departure. Hence, mechanistic understanding represents a fundamental step in designing the strategy for a chemical's development, since it will act as a driver for the design and measurement of specific endpoints in subchronic toxicity studies.

6.7. Use of a toolbox for measuring KEs

In the mechanism-based approach, perturbation of relevant MOAs may be detected through the use of a toolbox, *i.e.* combinations of *in silico* tools and *in vitro* assays and, where deemed necessary, short-term *in vivo* methods. Not all KEs involved may need to be measured to elucidate the MOA; instead, a subset of KEs may provide the required weight-of-evidence, with minimal to no uncertainty. The tools and methods need to specifically address molecular initiating events, KEs and/or specific intrinsic properties covering critical steps of the carcinogenic process but may not necessarily be linked directly to the apical endpoint. Given that tumour formation can occur in virtually any tissue or organ, the toolbox will probably comprise *in vitro* assays using different cell types. Some of these tools and methods may already be commonly used to fulfil existing data requirements. However, QSAR (quantitative structure–activity relationships) models and assays such as high-throughput screening *in vitro* assays from the ToxCast program (Chiu et al., 2018; Judson et al., 2010; Liu et al., 2017; Sipes et al., 2017) are also considered valuable, even though these may sometimes provide 'leads' rather than definitive MOAs. Insight into likely onset of (networks of) MOAs could also be obtained from (high-throughput) toxicogenomics approaches (Bhat et al., 2013; Cheung et al., 2019), *e.g.* through application of weighted gene co-regulated network analyses (Sutherland 2016, 2018). Findings from such analyses would need to be confirmed by more extensive targeted testing. Furthermore, the toolbox should include assays required for determination of toxicokinetic properties. Dose selection and metabolic competency is important for each of the assays included in the toolbox, especially when comparing *in vitro* concentrations with *in vivo* dosage and their relationship to predicted human exposures. Tools and methods included in the toolbox may be used in a tiered approach, with each tier probably consisting of a battery of assays. For each of the assays, information on scientific basis, standardisation of protocols, performance (prediction models, predictive capacity, reproducibility, strengths and weaknesses, applicability domain) and use within a weight-of-evidence approach (acceptance, reliability and remaining uncertainties) should be available. The workshop participants encouraged the exchange of data from different sectors to help balance the data source and chemical space. Establishment of the toolbox will contribute to a substantial reduction of animal use and cost; however, criteria for how to evaluate the weight-of-evidence approach and apply expert judgement will be required.

6.8. Uncertainty

Evaluation of uncertainty factors should be conducted where possible and where considered meaningful. Such analysis is relevant to exposure, toxicokinetic properties and assays used to determine

perturbation of relevant MOAs, including read-across or QSAR applications. The uncertainty analysis should also consider the possibility that alternative MOA(s) exists for which lines of evidence were not explored. One of the remaining topics for discussion is whether a higher level of confidence is needed for negative conclusions (*i.e.* lack of carcinogenic potential) based on this approach; ideally a negative outcome should be as reliable as a positive outcome.

7. Complementary activities

In addition to this EPAA project and the abovementioned ICH project, other international activities to develop alternative approaches to cancer risk assessment of NGTXC have been initiated worldwide. These include the Organisation for Economic Co-operation and Development (OECD) project on the development of an Integrated Approach to the Testing and Assessment (IATA) for NGTXC, the US EPA effort to develop a risk-based weight-of-evidence approach for waiving chronic and carcinogenicity studies for agrochemicals, and ongoing work at the International Agency for Research on Cancer on key characteristics of carcinogens (Smith et al., 2016). For the first time, the 2019 workshop has brought together representatives from these ongoing initiatives (Fig. 1), to exchange views, benefit from insights gained in each of these groups, to add synergies and develop together a roadmap for the EPAA project for the next steps. A concise summary of the OECD and EPA initiatives is given below.

7.1. OECD activities on an IATA for non-genotoxic carcinogens

The OECD set up an expert working group in 2016 as a result of the discussion of the Working Group of National Coordinators for the Test Guidelines Programme (WNT) in 2014, in which it was recognised that the Cell Transformation Assay using Syrian Hamster Embryo had limitations that were considered at that time to impede its regulatory acceptance as an OECD Test Guideline to address NGTXC, and that a more comprehensive toolbox of tests addressing their mechanisms was needed. The toolbox of mechanistic assays should be applicable for regulatory decision-making across all chemical production and use sectors. While some sectors, such as the agrochemical sector, under regulatory jurisdiction requirements can conduct cancer bioassays, other sectors have regulatory restrictions to do so, and often, after mutagenicity and genotoxicity testing has been conducted, there is no further testing for non-genotoxic carcinogenicity endpoints. This is a critical gap in protecting public health from chemical carcinogens. Therefore, a Steering Group was established at the WNT 2014 meeting to develop a 'thought starter' that could be the basis for the development of an IATA to address non-genotoxic carcinogenicity (Jacobs et al., 2016). The IATA would integrate and weigh all available data for hazard assessment purposes, in which the Cell Transformation Assay, together with other relevant assays, could fit. A review of the main "hallmarks of carcinogenicity" and associated MOAs were identified to better understand the biological processes associated with cancer (Jacobs et al., 2016). These were reorganised in different conceptual layers according to levels of biological organization. This was considered a pragmatic way of encompassing different theories on cancer, with a focus on key commitment steps but incorporating the transition from adaptive to mal-adaptive physiology, such that public health protection from NGTXC could be better enacted. The IATA would also accommodate different assay blocks and is ultimately intended to be applicable across sectors and with global regulatory requirements. So far, there are 13 assay building blocks, of which some address early to mid KEs of MOAs relevant for non-genotoxic carcinogenicity, including but not limited to receptor binding and cell proliferation. In order to obtain more relevant assays for different parts of the IATA, an OECD call for *in vitro* assays was made in 2018 (which is still open, see Supplementary Table 2 and specific assay needs in Jacobs et al. (2020)). The assays are being assessed and prioritised according to their state of standardisation and validation in relation to defined criteria. Based on the lessons learnt thus far, the conceptual IATA has now advanced to the development of a consensus IATA backbone framework with more mechanistically comprehensive assays from each block (Jacobs et al., 2020). There will be further OECD expert group meetings before a consolidated draft IATA guidance document can be submitted to the WNT for approval.

7.2. US EPA efforts on a weight-of-evidence risk-based waiver for carcinogenicity studies of agrochemicals

The US EPA published a guideline in May 2013, which describes the data/information needed for different endpoints of acute toxicity via the oral, dermal and inhalation routes, eye and skin irritation, skin sensitization, as well as acute and delayed neurotoxicity (Craig et al., 2019; US EPA, 2013; US EPA, 2013). The guideline ensures there is sufficient information to reliably support registration decisions that are protective of public health and the environment, while avoiding unnecessary use of time and resources, data generation costs, and animal testing. The US EPA waiver program started in 2011 and, since then, the US EPA has accepted a number of study waivers, including chronic and carcinogenicity studies (Craig et al., 2019). Currently, out of ~1500 requests across a range of study types, the US EPA has accepted 1300 waivers, suggesting that by waiving studies, half a million fewer animals, including test guideline and preliminary studies, were used. In

Fig. 1. International activities to develop alternative approaches to cancer risk assessment include initiatives from EPAA, OECD, IARC, ICH and US EPA. Representatives from these ongoing initiatives were brought together in the EPAA workshop.

alignment with the US EPA waiver program a collaboration entitled Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP), led by experts from the PETA International Science Consortium Ltd. (PETA-ISC), government, and industry was formed to address the opportunity to waive the chronic cancer bioassay for new pesticide registration. A problem formulation meeting was held with participants from the US EPA, National Toxicology Program (NTP), agrochemical and pharmaceutical industries, academia and NGOs (Sauve-Ciencewicki et al., 2019). This resulted in the problem statement that the bioassay is being conducted for pesticides where it is not always needed for assessment of human cancer hazard. In answer to the problem statement ReCAAP determined that carcinogenicity waiver requests are most appropriate where a strong exposure argument can be provided, as well as thorough information on chemical class (read-across), and an understanding of the MOA in relation to human relevance. The specific aspects considered were: use, classification, metabolism, and results of studies, including *in vitro* and repeated dose studies and mechanistic information. As a result, a decision tree based on Cohen et al. was used to evaluate for carcinogenicity potential and to inform a risk-based weight-of-evidence framework for cancer assessment that is applicable to a broad range of agrochemicals (Cohen et al., 2019).

8. Roadmap

Based on the discussions surrounding the case studies and considering the requirements for an alternative approach to cancer risk assessment, workshop participants agreed that the proposed approach is worthy of exploration and development for agrochemicals. Combining innovative tools and test methods with current knowledge of mechanisms involved in carcinogenesis will enable prediction of carcinogenic potential using a much smaller number of experimental animals, while also ensuring that public health protection needs are adequately addressed. Consequently, different aspects of the mechanism-based approach have been identified for further development.

8.1. Fit-for-purpose and flexible

The new approach should be effective yet flexible: it should be applicable for different regulatory purposes and accommodate different priorities and global regulatory contexts. At the same time, there needs to be agreement on how a mechanism-based approach is put into practice for a specific purpose. Therefore, for each regulation, application of the approach should be well-defined, including decision criteria for evaluating the weight-of-evidence. From an EPAA-perspective, the initial focus is on the agrochemical sector, but there is potential applicability to other regulations/purposes, such as REACH and CLP, provided the approach will be accepted for classifying substances for carcinogenicity. By continuing the dialogue with similar initiatives and a wide group of stakeholders, we are aiming for global harmonisation of requirements to maximise 3Rs benefits, cost- and time-effectiveness.

8.2. Human-relevant MOAs

Acceptance and implementation of the mechanism-based approach will be greatly facilitated if the approach encompasses key aspects of all MOAs that are considered relevant for non-genotoxic carcinogenesis. One of the next steps is therefore to further investigate the set of carcinogenic substances for which the MOA underlying tumour formation could not yet be identified (Heusinkveld et al., 2020). A critical review of the data available for these substances will also yield a mapping of possible further needs to unravel the MOAs involved. Another source of information for identifying MOAs that are relevant to chemical-induced carcinogenesis are data for human cancers which do not manifest in animal models. Subsequently, consensus needs to be reached on the MOAs to be included in the new approach. One may argue that these MOAs should be limited to those that are considered human relevant. At the same time, inclusion of a larger set of MOAs involved in carcinogenesis, irrespective of current knowledge on their (lack of) human relevance, may facilitate uptake by the regulatory community as it may provide stronger correlations to findings that currently need to be reported (*i.e.* animal data). In due time, with progressing insight into the human relevance of each MOA, the set of MOAs will be refined to include only those that are proven to be truly human relevant. Finally, for each of the MOAs included in the mechanism-based approach, consensus needs to be reached on the weight-of-evidence that will be required to demonstrate that a chemical under study is likely (or not) to trigger a particular MOA.

8.3. 3Rs-based toolbox

Once the relevant MOAs and their KEs have been agreed upon, suitable assays for measuring these quantitatively (where possible) need to be identified. For this, we will closely collaborate with the OECD's expert group on the IATA for NGTXC, since a systematic evaluation of relevant assays using ranking parameters and a quantitative scoring system is currently being conducted by that team (Jacobs et al., 2020). The set of selected assays preferably consists of non-animal test methods and comprises, where possible, assays that are validated (or are likely candidates to be eventually developed and adopted as OECD Test Guidelines).

The abovementioned efforts will result in a qualitative approach for predicting both the presence and absence of carcinogenic potential of chemicals (Fig. 2). The negative and positive predictive value of this approach needs to be evaluated using a selected set NGTXC and non-carcinogens with different MOAs. Once it has been proven to be sufficiently robust, a weight-of-evidence based waiver program for agrochemicals could be designed to evaluate the approach for a larger group of chemicals and to familiarize end-users.

9. Summary and next steps

The EPAA project aims to develop and evaluate a mechanism-based approach that relies on a combination of short-term *in vivo* toxicity studies together with mechanistic information. New technologies and integrating new testing models and tools into a decision-tree will enable this approach. As a result of the availability of mechanistic tools and assays, as well as a much better understanding of the processes involved in carcinogenicity and the (sustained) doses at which they occur, it is believed that tumour formation in different organs can be predicted using mechanistic and cellular endpoints, rather than the morphological endpoints measured in carcinogenicity studies that are demanding in terms of animal use, cost and time. The participants of the workshop from different sectors agreed that this approach is worth pursuing and complements other international initiatives, such as those driven by OECD and US EPA.

Take-home messages and conclusions from various break-out group and plenary discussions indicated a clear and positive consensus that the proposed mechanism-based approach is a way forward. The four case studies presented showed that while fine-tuning is needed, there were no significant data gaps in the KEs and MOAs known to be involved in these agrochemical-induced carcinogenic processes. Participants were confident that a toolbox of *in silico*, *in vitro*, and short-term *in vivo* models could in time be used to identify the different MOAs involved in carcinogenesis and define how the toolbox can be incorporated into the carcinogenicity assessment framework.

This project is an important step towards the longer-term goal to completely change the carcinogenicity assessment paradigm. Participants considered the workshop as highly valuable to have constructive discussions with different stakeholders and reach consensus on the roadmap – moving away from the descriptive carcinogenicity assay towards more mechanistic-based approaches to the prediction of carcinogenesis. The hope is that in time global harmonisation can be

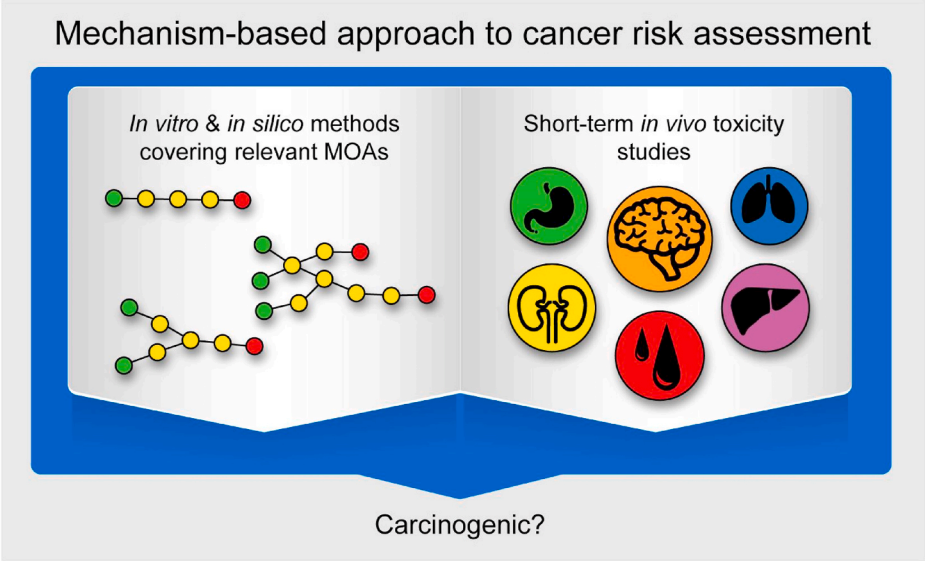

Fig. 2. Schematic overview of the mechanism-based approach, which combines data from a toolbox of targeted test methods for detecting MOAs underlying non-genotoxic carcinogenicity with information from short-term *in vivo* toxicity studies.

achieved, which requires consensus on the mechanisms involved in carcinogenesis, their relevance to humans, and on how to assess whether or not a particular MOA is operative for a particular chemical. While it was recognised that the proposed mechanism-based approach must meet regulatory requirements and that further work will be required, workshop participants considered this integrated method to be a realistic approach that could contribute to finally gaining regulatory acceptance globally. Ultimately, the movement towards a more scientifically relevant approach should be able to appropriately safeguard human health using up-to-date scientific and technological advances that have developed since the dawn of the traditional carcinogenicity assay 60 years ago.

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Declaration of competing interest

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References

Ames, B.N., Gold, L.S., 1990. Too many rodent carcinogens: mitogenesis increases mutagenesis. Science 249, 970–971.

Anisimov, V.N., Ukraintseva, S.V., Yashin, A.I., 2005. Cancer in rodents: does it tell us about cancer in humans? Nat. Rev. Canc. 5, 807–819.

Annys, E., Billington, R., Clayton, R., Bremm, K.D., Graziano, M., McKelvie, J., Ragan, I., Schwarz, M., van der Laan, J.W., Wood, C., Oberg, M., Wester, P., Woodward, K.N., 2014. Advancing the 3Rs in regulatory toxicology - carcinogenicity testing: scope for harmonisation and advancing the 3Rs in regulated sectors of the European Union. Regul. Toxicol. Pharmacol. 69, 234–242.

Bhat, V.S., Hester, S.D., Nesnow, S., Eastmond, D.A., 2013. Concordance of transcriptional and apical benchmark dose levels for conazole-induced liver effects in mice. Toxicol. Sci. 136, 205–215.

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, 781–792.

Bourcier, T., McGovern, T., Stavitskaya, L., Kruhlak, N., Jacobson-Kram, D., 2015. Improving prediction of carcinogenicity to reduce, refine, and replace the use of experimental animals. J Am Assoc Lab Anim Sci 54, 163–169.

Cheung, C., Jones-McLean, E., Yauk, C., Barton-Maclaren, T.S., Boucher, S., Bourdon-Lacombe, J., Chauhan, V., Gagné, M., Gillespie, Z., Halappanavar, S., Honeyman, M., Jones, S.R., Labib, S., MacAulay, J., Moore, J., Paquette, M., Petronella, N., Semalulu, S., Situ, D., Slot, A., Vespa, A., Woodland, C., 2019. Evaluation of the use of toxicogenomics in risk assessment at health. CanadaFebruary. https://doi.org/10.1016/j.cotox.2019.02.005.2019.

Chiu, W.A., Guyton, K.Z., Martin, M.T., Reif, D.M., Rusyn, I., 2018. Use of high-throughput in vitro toxicity screening data in cancer hazard evaluations by IARC Monograph Working Groups. ALTEX 35, 51–64.

Cohen, S.M., Boobis, A.R., Dellarco, V.L., Doe, J.E., Fenner-Crisp, P.A., Moretto, A., Pastoor, T.P., Schoeny, R.S., Seed, J.G., Wolf, D.C., 2019. Chemical carcinogenicity revisited 3: risk assessment of carcinogenic potential based on the current state of knowledge of carcinogenesis in humans. Regul. Toxicol. Pharmacol. 103, 100–105.

Cohen, S.M., Ellwein, L.B., 1991. Genetic errors, cell proliferation, and carcinogenesis. Canc. Res. 51, 6493–6505.

Craig, E., Lowe, K., Akerman, G., Dawson, J., May, B., Reaves, E., Lowit, A., 2019. Reducing the need for animal testing while increasing efficiency in a pesticide regulatory setting: lessons from the EPA office of pesticide programs' hazard and science policy council. Regul. Toxicol. Pharmacol. 108, 104481.

EC, 2008. European Commission (EC). EU Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Text with EEA relevance). Official Journal L 1–739.

EC, 2009. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EC and 91/414/EC. Official Journal L 309, 1–50.

EC, 2013. European Commission regulation (EU) no 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with regulation (EC) no 1107/2009 of the European parliament and of the council concerning the placing of plant protection products on the market. Off. J. Eur. Union 93, 85–152.

EFSA, 2011. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 9, 2379.

Elcombe, C.R., Peffer, R.C., Wolf, D.C., Bailey, J., Bars, R., Bell, D., Cattley, R.C., Ferguson, S.S., Geter, D., Goetz, A., Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C.J., Schoeny, R., Xie, W., Lake, B.G., 2014. Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: a case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit. Rev. Toxicol. 44, 64–82.

Goodman, J.I., 2018. Goodbye to the bioassay. Toxicol Res (Camb). 7, 558–564.

Gottmann, E., Kramer, S., Pfahringer, B., Helma, C., 2001. Data quality in predictive toxicology: reproducibility of rodent carcinogenicity experiments. Environ. Health Perspect. 109, 509–514.

Haseman, J.K., Hailey, J.R., Morris, R.W., 1998. Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. Toxicol. Pathol. 26, 428–441.

Hernandez, L.G., van Steeg, H., Luijten, M., van Benthem, J., 2009. Mechanisms of non-genotoxic carcinogens and importance of a weight of evidence approach. Mutat. Res. 682, 94–109.

Heusinkveld, H., Braakhuis, H., Gommans, R., Botham, P., Corvaro, M., van der Laan, J. W., Lewis, D., Madia, F., Manou, I., Schorsch, F., Wolterink, G., Woutersen, R., Corvi, R., Mehta, J., Luijten, M., 2020. Towards a Mechanism-Based Approach for the Prediction of Non-genotoxic Carcinogenic Potential of Agrochemicals. Submitted for publication.

ICH, 2013. International Conference on Harmonization (ICH). 2013. Regulatory Notice Document: proposed change to rodent carcinogenicity testing of pharmaceuticals. Available at: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ich-guideline-s1-regulatory-notice-changes-core-guideline-rodent-carcinogenicity-testing_en.pdf. (Accessed 6 May 2020).

Jacobs, M.N., Colacci, A., Louekari, K., Luijten, M., Hakkert, B.C., Paparella, M., Vasseur, P., 2016. International regulatory needs for development of an IATA for non-genotoxic carcinogenic chemical substances. ALTEX 33, 359–392.

Jacobs, M.N., Colacci, A., Corvi, R., Vaccari, M., Aguila, M.C., Corvaro, M., Delrue, N., Desaulniers, D., Ertych, N., Jacobs, A., Luijten, M., Madia, F., Nishikawa, A., Ogawa, K., Ohmori, K., Paparella, M., Sharma, A.K., Vasseur, P., 2020. Chemical carcinogen safety testing: OECD expert group international consensus on the development of an integrated approach for the testing and assessment of chemical non-genotoxic carcinogens [published online ahead of print, 2020 Jun 27]. Arch. Toxicol. https://doi.org/10.1007/s00204-020-02784-5, 2020.

Jacobson-Kram, D., Sistare, F.D., Jacobs, A.C., 2004. Use of transgenic mice in carcinogenicity hazard assessment. Toxicol. Pathol. 32 (Suppl. 1), 49–52.

Jaeger, B.R., 1984. Pesticide assessment guidelines: subdivision F: hazard evaluation: human and domestic animals (revised edition). In: Washington, D.C. (Ed.), Office of Pesticides and Toxic Substances, U.S. E.P.A. EPA No. 54019-84-01.

Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen, H.M., Reif, D.M., Rotroff, D.M., Shah, I., Richard, A.M., Dix, D.J., 2010. In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. EHP (Environ. Health Perspect.) 118, 485–492.

Liu, J., Patlewicz, G., Williams, A.J., Thomas, R.S., Shah, I., 2017. Predicting organ toxicity using in vitro bioactivity data and chemical structure. Chem. Res. Toxicol. 30, 2046–2059.

Luijten, M., Olthof, E.D., Hakkert, B.C., Rorije, E., van der Laan, J.W., Woutersen, R.A., van Benthem, J., 2016. An integrative test strategy for cancer hazard identification. Crit. Rev. Toxicol. 46, 615–639.

Marty, M.S., Papineni, S., Coady, K.K., Rasoulpour, R.J., Pottenger, L.H., Eisenbrandt, D. L., 2015. Pronamide: weight of evidence for potential estrogen, androgen or thyroid effects. Regul. Toxicol. Pharmacol. : RTP (Regul. Toxicol. Pharmacol.) 72, 405–422.

Meek, M.E., Palermo, C.M., Bachman, A.N., North, C.M., 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, 595–606.

Meek, M.E.B., Bucher, J.R., Cohen, S.M., Dellarco, V., Hill, R.N., Lehman-McKeeman, L. D., Longfellow, D.G., Pastoor, T., Seed, J., Patton, D.E., 2003. A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33, 591–653.

OECD, 2018. Test No. 451. In: Carcinogenicity Studies, OECD Guidelines for the Testing of Chemicals, Section, vol. 4. OECD Publishing, Paris. https://doi.org/10.1787/9789264071186-en.

Paparella, M., Colacci, A., Jacobs, M.N., 2017. Uncertainties of testing methods: what do we (want to) know about carcinogenicity? ALTEX 34, 235–252.

Papineni, S., Marty, M.S., Rasoulpour, R.J., LeBaron, M.J., Pottenger, L.H., Eisenbrandt, D.L., 2015. Mode of action and human relevance of pronamide-induced rat thyroid tumors. Regul. Toxicol. Pharmacol. : RTP (Regul. Toxicol. Pharmacol.) 71, 541–551.

Peffer, R.C., LeBaron, M.J., Battalora, M., Bomann, W.H., Werner, C., Aggarwal, M., Rowe, R.R., Tinwell, H., 2018. Minimum datasets to establish a CAR-mediated mode of action for rodent liver tumors. Regul. Toxicol. Pharmacol. : RTP (Regul. Toxicol. Pharmacol.) 96, 106–120.

Sauve-Ciencewicki, A., Davis, K.P., McDonald, J., Ramanarayanan, T., Raybould, A., Wolf, D.C., Valenti, T., 2019. A simple problem formulation framework to create the right solution to the right problem. Regul. Toxicol. Pharmacol. 101, 187–193.

Sipes, N.S., Wambaugh, J.F., Pearce, R., Auerbach, S.S., Wetmore, B.A., Hsieh, J.H., Shapiro, A.J., Svoboda, D., DeVito, M.J., Ferguson, S.S., 2017. An intuitive approach for predicting potential human health risk with the Tox21 10k library. Environ. Sci. Technol. 51, 10786–10796.

Sistare, F.D., Morton, D., Alden, C., Christensen, J., Keller, D., Jonghe, S.D., Storer, R.D., Reddy, M.V., Kraynak, A., Trela, B., Bienvenu, J.G., Bjurstrom, S., Bosmans, V., Brewster, D., Colman, K., Dominick, M., Evans, J., Hailey, J.R., Kinter, L., Liu, M., Mahrt, C., Marien, D., Myer, J., Perry, R., Potenta, D., Roth, A., Sherratt, P., Singer, T., Slim, R., Soper, K., Fransson-Steen, R., Stoltz, J., Turner, O., Turnquist, S., van Heerden, M., Woicke, J., DeGeorge, J.J., 2011. An analysis of pharmaceutical experience with decades of rat carcinogenicity testing: support for a proposal to modify current regulatory guidelines. Toxicol. Pathol. 39, 716–744.

Smith, M.T., Guyton, K.Z., Gibbons, C.F., Fritz, J.M., Portier, C.J., Rusyn, I., DeMarini, D. M., Caldwell, J.C., Kavlock, R.J., Lambert, P.F., Hecht, S.S., Bucher, J.R., Stewart, B. W., Baan, R.A., Cogliano, V.J., Straif, K., 2016. Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. Environ. Health Perspect. 124 (6), 713–721.

Smith, C.J., Perfetti, T.A., 2018. The "false-positive" conundrum in the NTP 2-year rodent cancer study database. Toxicology Research and Application 2, 2397847318772839.

Sutherland, J.J., Webster, Y.W., Willy, J.A., Searfoss, G.H., Goldstein, K.M., Irizarry, A. R., Hall, D.G., Stevens, J.L., 2018. Toxicogenomic module associations with pathogenesis: a network-based approach to understanding drug toxicity. Pharmacogenomics J. 18 (3), 377–390.

Sutherland, J.J., Jolly, R.A., Goldstein, K.M., Stevens, J.L., 2016. Assessing concordance of drug-induced transcriptional response in rodent liver and cultured hepatocytes. PLoS Comput. Biol. 12 (3), e1004847.

US EPA, 1986. Risk Assessment Forum. Guidelines for mutagenicity risk assessment. Federal Register 51 (185), 34006–34012.

US EPA, 2013. Office of Pesticide Programs. Guiding principles for data requirements. Washington, DC 20460.

US EPA, 2013. Office of Pesticide Programs. Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies. https://www.epa.gov/sites/production/files/2014-02/documents/part158-tox-data-requirement.pdf.

van der Laan, J.W., Kasper, P., Silva Lima, B., Jones, D.R., Pasanen, M., 2016a. Critical analysis of carcinogenicity study outcomes. Relationship with pharmacological properties. Crit. Rev. Toxicol. 46 (7), 587–614.

van der Laan, J.W., Buitenhuis, W.H., Wagenaar, L., Soffers, A.E., van Someren, E.P., Krul, C.A., Woutersen, R.A., 2016b. Prediction of the carcinogenic potential of human pharmaceuticals using repeated dose toxicity data and their pharmacological properties. Front. Med. 3, 45.

US EPA, 2016. The Frank R. Lautenberg Chemical Safety for the 21st Century Act. https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/frank-r-lautenberg-chemical-safety-21st-century-act. 2016.

van der Laan, J.W., Bourcier, T., Cavaliero, T., McGovern, T., Nishikawa, A., Nonaka, M., Ogawa, K., Pasanen, M., Ebere, G., 2019. The ICHS1 regulatory testing paradigm of carcinogenicity in rats. Status Rep. Retrieved from https://database.ich.org/sites/default/files/S1_StatusReport_2019_0802.pdf. 2019.