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MEETING REPORTS

The use of adverse outcome pathways in the safety evaluation of food additives

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Abstract

In the last decade, adverse outcome pathways have been introduced in the fields of toxicology and risk assessment of chemicals as pragmatic tools with broad application potential. While their use in the pharmaceutical and cosmetics sectors has been well documented, their application in the food area remains largely unexplored. In this respect, an expert group of the International Life Sciences Institute Europe has recently explored the use of adverse outcome pathways in the safety evaluation of food additives. A key activity was the organization of a workshop, gathering delegates from the regulatory, industrial and academic areas, to discuss the potentials and challenges related to the application of adverse outcome pathways in the safety assessment of food additives. The present paper describes the outcome of this workshop followed by a number of critical considerations and perspectives defined by the International Life Sciences Institute Europe expert group.

Keywords Adverse outcome pathway · Food additive · Safety evaluation

Abbreviations

3Rs	Replacement, reduction and refinement
AOP(s)	Adverse outcome pathway(s)
EFSA	European Food Safety Authority
ILSI	International Life Sciences Institute
KE(s)	Key event(s)
MIE(s)	Molecular initiating event(s)
OECD	Organization for Economic Cooperation and Development
PBK	Physiologically-based kinetic
QIVIVE	Quantitative in vitro-to-in vivo extrapolation
US EPA	United States Environmental Protection Agency

Introduction

Toxicology, and hence chemical safety evaluation practice, have drastically changed over the past few decades. In fact, this area is still transitioning from classical toxicology, focusing on measuring apical endpoints of toxicity in animal

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models, to animal-free and more human-relevant toxicology, relying on mechanistic information. This paradigm shift has been facilitated by the introduction of the concept of adverse outcome pathways (AOPs) (Ankley et al. 2010), which are gaining momentum worldwide. This particularly holds true for the cosmetics sector, where a full animal testing ban has been imposed by European legislation a number of years ago (EU 2009). In this respect, tiered animal-free approaches for the safety evaluation of cosmetic ingredients, driven by exposure and mechanistic information, have been developed (Dent et al. 2018; Desprez et al. 2018). This has stimulated other industries also to explore the application of such strategies for chemical risk assessment more generally, thus complying with the 3Rs principle (i.e. replacement, reduction and refinement of animal experimentation) (Russell and Burch 1959). Among these is the food additive area, which still largely relies on testing in animal models for hazard identification purposes. In this context, the task force of "New approaches to chemical risk assessment for foods and food ingredients" at the International Life Sciences Institute (ILSI) Europe intensively monitors and evaluates such non-animal strategies for implementation in current chemical risk assessment practice. In 2017, this ILSI Europe task force established an expert group to specifically explore the use of AOPs in the safety evaluation of food additives. One of the key activities of this expert group was the organization of a workshop aimed at triggering an in-depth discussion on potentials and challenges related to the application of AOPs in the food industry. This workshop was organized on 26–27 February 2019 in Brussels, Belgium and gathered approximately 30 experts with an academic, industrial or regulatory background. The present manuscript describes the major outcomes and recommendations from this workshop, and as such is a follow-up of a study of the ILSI expert group that characterized the coverage of critical effects relevant in the safety evaluation of food additives by AOPs (Kramer et al. 2019). The results of the workshop are described followed by some critical considerations and perspectives from the members of the expert group on the use of AOPs in the safety evaluation of food additives.

Outcome of the workshop

ILSI Europe's workshop on the use of AOPs in the safety evaluation of food additives comprised a combination of expert lectures and break-out group discussions. Expert lectures covered AOP topics from a number of different perspectives, including case studies from the regulatory, cosmetics and pesticide sectors. In pesticide safety, the United States Environmental Protection Agency (US EPA) uses AOPs to help establish common mechanism groups for cumulative risk assessment, and the European Food Safety Authority (EFSA) is using AOPs to understand mechanisms of toxicity as part of safety evaluation. Regulatory changes in the cosmetics industry have led the way in forcing the hand of regulators and toxicologists to champion new approaches, with AOPs seeing use in hazard identification/characterization to help the identification of the critical effect on which to base the determination of the point-of-departure. These examples show the value of good data, AOP development and regulatory acceptance in the deployment of new approaches to safety evaluation.

During the break-out sessions, the workshop participants were asked to consider several questions (Table 1). The workshop participants agreed that AOP development and usage is still predominantly an academic exercise, as a means to organize molecular mechanisms in a toxicologically relevant pathway. However, it was emphasized that AOPs share many conceptual similarities with modes-of-action, the application of which in regulatory risk assessment is well established. Hence, some of the lessons learned will be of value in the introduction of AOPs of this purpose. It was also noted that the concept of AOPs is already well ingrained into the language of environmental risk assessment and the safety evaluation of cosmetic ingredients. In both cases, AOPs are applied as part of a tiered assessment approach and a means of prioritizing tests.

The workshop participants classified the use of AOPs in non-food sectors as either part of a top-down or bottom-up

Table 1 Questions covered by the attendees of the ILSI Europe workshop "The use of AOPs for the safety evaluation of food additives" organized on 26–27 February 2019 in Brussels-Belgium

How are you currently using the AOP framework or how do you envision to use it in the future in your field of work?
(How) could the food industry similarly apply the AOP concept to improve food safety assessment?
At what point in the hazard identification of a food additive do you see a potential to use the AOP concept?
Can the use of AOPs contribute to replacing animal studies in the hazard assessment of food additives?
How would you apply the AOP framework for the different types of food safety assessment from screening to regulation?
What do AOPs need to contain to be useful in the safety assessment of food additives?
What are the shortcomings and major gaps in knowledge hampering the application of AOPs in the safety assessment of food additives?
What is needed to address these gaps?
How can risk assessment methodologies be adapted to best use AOPs?

approach. The former approach entails using AOPs to design in vitro test batteries for testing (new) chemical entities for their ability to perturb the pathways and provide evidence for a likelihood of a particular adverse health outcome to occur. An example of such an approach is the use of the skin sensitization AOP for safety testing of cosmetic ingredients. In vitro tests, including the direct peptide reactivity, anti-oxidant/electrophile response element nuclear erythroid 2-related factor 2 and human cell line activation test assays, cover specific key events (KEs) within this AOP, for which specific test guidelines at the Organization for Economic Cooperation and Development (OECD) have been developed. These assays allow to assess the sensitizing potential of chemicals by integrating the results of each assay in a weight-of-evidence approach. Such an approach is also used to sort chemicals according to their perturbation potential of KEs and use the assay results to justify read-across. In other words, AOPs are a means of performing read-across by toxicological mechanism. In doing so, it allows for a strategy to assess what tests to perform for each individual chemical.

The bottom-up approach is increasingly applied in academic and industrial settings. It entails using AOPs to discern and classify molecular mechanisms of toxicity. In this context, safety evaluations move away from assessing whether exposure to a particular chemical is associated with a particular toxic endpoint, such as hepatotoxicity, but rather how to integrate various pieces of information, from bioinformatics, biomonitoring data to animal test results, into an integrated understanding of how multiple stressors affect the overall health of specific populations. Examples of these can be seen in ecotoxicology, including the development of AOPs leading to chronic fish toxicity focused on directly experimentally measurable KEs shared by multiple pathways, in casu impaired swimbladder inflation (Villeneuve et al. 2014). AOPs conveniently provide a mechanistic underpinning that is pivotal in understating whether the adverse outcomes observed with particular chemical exposures in animal toxicity studies are of relevance to humans (Bal-Price and Meek 2017; Jeong and Choi 2017).

The workshop participants agreed that AOPs could be used in a similar way in the food industry, as similar principles of evaluation apply. Both the bottom-up and top-down approaches are considered to be useful for performing safety evaluation of food additives. The use of AOPs in the safety evaluation process is expected to occur at the hazard identification stage, where in vitro and in silico assays flag possible perturbations of KEs of known AOPs. This, in turn, may help in devising a test strategy for hazard characterization (i.e. dose–response assessment) of new chemical entities from which points-of-departures are derived. AOPs can also be used to identify mechanisms of toxicity associated with adverse outcomes found in toxicity tests, and thus help determine the relevance of the toxic effect to humans. In addition, information from AOP perturbations, such as from screenings, allows for incorporation of additional endpoints, such as induction of biotransformation enzymes, to explain liver effects (Kang et al. 2018; Nepelska et al. 2017). For example, when it is known that the tumorigenesis of a chemical in rodents is related to peroxisome proliferator-activated receptor alpha activation, using these data for risk assessment is questionable, since the pathway is not relevant in humans (Corton et al. 2018). AOPs allow for a better understanding of species specificity of adverse health effects and thus allow for the selection of appropriate animal species for testing (i.e. refinement). AOPs also allow for carrying out mechanistically-based read-across approaches and provide arguments for performing limited targeted animal testing or, ideally, avoiding them. In sectors where in vivo toxicity data are still a regulatory requirement, such as the pharmaceutical industry, the complete replacement of animal testing would first require appropriate changes in policy and legislation. Nevertheless, with the projected gain in efficiency in testing provided by the use of AOPs, animal testing is likely to be reduced and refined.

A specific challenge in considering the use of AOPs in the safety evaluation of food additives is the lack of data to develop food-industry relevant AOPs. While the pharmaceutical industry has ample clinical studies to relate specific human health outcomes to in silico, in vitro and preclinical data, such data are often lacking for food additives. AOPs relevant to food additives are likely to vary from the pathways common in other industries, such as for endpoints-like gastrointestinal tract irritation, an area where many of the molecular mechanisms are currently unknown. An AOP to assist in understanding this would be of enormous value to the food industry. As analyzed by Kramer et al. (2019) as part of the ILSI Europe expert group activities, the adverse outcomes associated with food additives include a number of non-specific, late-onset and/or local effects, such as body weight changes, for which no AOPs have been developed. Particularly molecular initiating events (MIEs) and early KEs are currently insufficiently identified, and chemicals will likely perturb numerous MIEs simultaneously when point-of-departure values are high. Given the non-specific nature of the adverse outcomes, it is also essential that KEs and measurable intermediate effects associated with multiple pathways are identified. By identifying these "superhubs", directed in vitro and in silico testing strategies could be applied to inform multiple AOPs rapidly. Moreover, it is essential to quantify AOPs and consider networks when using them for the safety evaluation of food additives. Changes in biomarkers of a plethora of KEs in bioassays could be quantitatively associated with high human exposures leading to multiple adverse outcomes. This requires the

integration of toxicokinetic processes, modulating factors and feedback loops in AOPs.

A final consideration from the workshop participants was the desire for an overarching AOP strategy on an international level integrating expert researchers, toxicologists and regulators. This could help to define the criteria required to ensure a food additive is safe and guide AOP development in both a qualitative and quantitative respect. Construction of AOPs is a challenging and time-consuming task. While many biological mechanisms are currently well understood, AOPs simply have not been constructed for these yet. Medical doctors have much of the required knowledge to assist in AOP development, thus AOP developers need to get them on board. Ultimately, an AOP strategy for food additive safety must convince those in the industry and regulators of its value through reduced cost, but also must be scientifically sound and human-relevant. Incorporation of more in vitro and in silico methods can certainly be faster than current methods as well, helping the industry to keep up with demands (Ehrlich et al. 2015).

In conclusion of the workshop, several key aspects of AOP development and deployment require further consideration to be used in food additive safety evaluation, and perhaps more generally. For AOPs to be used in food additive safety evaluation, there will need to be a focus on AOPs relevant to those chemicals, both in the case of optimizing existing AOPs to make them fit-for-purpose and for developing new AOPs for adverse health effects that could be relevant for food additives, such as food allergies/intolerances and gastrointestinal tract irritation. These AOPs can be combined with others to identify "superhub" KEs that can allow for the evaluation of multiple pathways at once, improving the efficiency of testing. To aid in the development of these AOPs, a better level of mechanistic understanding is required and one source of this knowledge could be through clinicians. EFSA guidance and further case studies are needed to explore the use of AOPs in the food sector, and in the future, these AOPs need to be quantitative to provide the necessary information for food safety assessment. Finally, the ILSI Europe expert groups on AOPs and ToxCast data should be linked to establish overlap and as a foundation for the further developments outlined above.

Critical considerations and perspectives

The outcome of the workshop clearly highlighted the importance of AOPs and the possibilities for their use in the process of safety evaluation of food additives. The workshop indicated that the use of AOPs in the safety evaluation process is at the moment most likely at the hazard identification stage. Thus, AOPs may help in defining critical effects, target organs and relevance of the adverse outcome for human risk assessment. This could be particularly useful considering that often the adverse effects seen at high-dose animal studies are of low relevance in the context of much lower human exposure levels or when the mechanism-of-action is species-specific. The use of exposure information is a key element to understand the correlation between in vitro concentrations and in vivo doses, and consequently the relevance to human exposure scenarios. Moreover, the development and use of human-relevant AOPs could aid the shift to a new paradigm for safety evaluation that does not necessarily rely on the extrapolation and/or on the prediction of animal data, but rather makes use of human-relevant mechanistic information, such as those derived from in vitro human cell-based assays and/or in silico approaches.

AOPs may also support the selection of adequate in vitro assays to detect KEs that are relevant for a specific mechanism-of-action. Furthermore, AOPs may facilitate read-across and provide support for the determination of relative potencies of related chemicals in a group based on a similar mode-of-action.

Inclusion of AOPs in a testing strategy for the hazard characterization (i.e. dose–response assessment) of new chemical entities to define points-of-departure appears less straightforward. In this context, the US EPA has recently published a risk-based tiered testing framework that combines computational modelling, high-throughput assays and AOPs to estimate points-of-departure for chemical safety assessment (Thomas et al. 2019). Points-of departure for risk assessment include, for example, no-observed-adverse-effect levels or benchmark-dose-lower-confidence limits usually derived from dose–response data from apical toxic endpoints. The use of the molecular/cellular endpoints for risk assessment, such as those reflecting KEs in an AOP, carries a number of challenges. First, it still needs to be established whether and at what level the perturbation seen in KEs is relevant for the prediction of the apical toxic endpoint (*i.e.* the adverse outcome). This would require the development and use of quantitative, not only qualitative, AOPs for systemic toxic endpoints, as described recently by Perkins et al. (2019), Zgheib et al. (2019), and Battistoni et al. (2019). Moreover, points-of-departure derived from in vitro bioassays reflecting KEs in an AOP cannot directly be converted into health-based reference values. Indeed, this requires translation of the in vitro concentration–response data to in vivo dose–response data taking kinetics into account. Although the workshop pointed to the importance of including kinetics in AOPs, this is presently limited to describing, for example, bioactivation as one of the KEs. The incorporation of exposure and toxicokinetic information into the AOP framework is one of the main challenges when considering the use of mechanistic data to derive a point-of-departure. This may best be achieved by combining AOPs with physiologically-based kinetic (PBK) models. Today,

many examples, some of those involving food chemicals, have been provided showing that the use PBK model-based reverse dosimetry for quantitative in vitro-to-in vivo extrapolation (QIVIVE) enables definition of points-of-departure for risk assessment (Levorato et al. 2019; Louisse et al. 2017). Thus far, this concept has not been used for AOPs specifically, although it could play a role in supporting the choice and relevance of the in vitro bioassay used to identify critical molecular endpoints. Another approach that is gaining popularity is the comparison of high-throughput bioactivity data, which could be representative for a specific MIE, with PBK-predicted human plasma concentrations (Dent et al. 2019; Wetmore et al. 2015). This type of approach is particularly promising as it is entirely focused on the assessment of human safety rather than trying to correlate and extrapolate animal toxicity data to the human situation.

The use of AOPs may also become relevant when there is a need for extending a validated QIVIVE-based prediction for a chemical for which toxicological data are lacking, but which are available for related chemicals. In such cases, AOPs may support that the chemicals act similarly, so that the QIVIVE approach proven valid for one chemical in the group may be used for related chemicals. Obviously, potential differences in kinetics should then still be taken into account.

The results of the workshop also clearly argued that lack of mechanistic knowledge may result in inadequate safety assessment. This is not a new observation, since information on mode-of-action often has been, and still is, essential in risk assessment, as can best be illustrated by some examples. A first example is the endpoint of carcinogenicity and the question of whether the underlying mode-of-action involves genotoxicity or not. Depending on the mode-of-action, the risk assessment is performed differently. For instance, given that no safe level of exposure can be anticipated for genotoxic carcinogens, no acceptable daily intake value can be established for these compounds, which are not allowed for use as food additives (EFSA 2005). Other examples where mode-of-action considerations were already an integral part of risk assessment before introduction of the AOP concept are in determining the relevance, or lack thereof, to humans of alpha 2u-globulin-related kidney toxicity observed only in male rats and not in female rats or humans (Doi et al. 2007), and rodent thyroid tumors following induction of thyroid hormone glucuronidation (Boobis et al. 2016). This also holds for the example presented during the workshop of hepatic tumorigenesis in rodents via peroxisome proliferator-activated receptor alpha activation, which is a pathway not relevant in humans (Corton et al. 2018). The advantage of the AOP initiative is in the international activity being coordinated by the OECD to provide a global repository of information and an AOP-based assessment strategy for chemicals.

A bottleneck highlighted by the workshop participants for the application of AOPs in the safety assessment of food additives might be the availability of relevant data to develop AOPs for these compounds, as opposed to, for example, drugs or pesticides. There may be several reasons underlying this situation, which may not be easily resolved. First, as shown by the study of Kramer et al. (2019), characterizing the coverage of critical effects relevant in the safety evaluation of food additives by AOPs, food additives are not designed to cause biological effects in humans. For only 22% of the food additives currently authorized in the European Union, a specific critical adverse effect has been identified (Kramer et al. 2019). This implies that how AOPs can be used as part of the standard battery of guideline requirements for safety testing of food additives needs detailed and careful assessment, and their use should be considered on a case-by-case basis. A possible application for the evaluation of new food additives, based on the framework proposed by the US EPA (Thomas et al. 2019), could be to run high-throughput screening assays across different cell lines targeting MIEs that lead to adverse outcomes that are commonly used for regulatory purposes. Based on the results, more specific tests could be performed to provide insight into the mode-of-action of the chemical. Another consideration is that, in contrast to drugs, food additives are not subject to risk–benefit consideration. Food additives will only be accepted when they do not pose a safety concern to the health of the consumers. This implies that including clinical input in the development of AOPs for food additives, as suggested during the workshop, may only be of use for certain endpoints. This may include, for example, allergy-related effects, since at present, allergy is an endpoint not considered suitable for defining health-based safety values for food additives. Allergens are rather regulated via adequate labelling, so that individuals who are allergic to a particular food additive can avoid exposure. It could be foreseen that for these types of adverse effects, the AOPs and model systems used for skin sensitization may prove to be of use.

In addition, the analysis of Kramer et al. (2019) revealed that the toxicological endpoints used for the safety assessment of food additives could be linked to a number of different AOPs. Therefore, understanding the specific mechanism-of-action leading to an adverse effect and, in case of chemicals acting via multiple mechanisms-of-action, the integration of information covered by different AOPs would be crucial to inform the risk assessment.

Finally, it is of interest to evaluate to what extent the workshop answered the questions put forward. The workshop provided views and perspectives on how the food industry could apply the AOP concept to improve the quality of their scientific dossiers, taking into account information on the mode-of-action to support relevance of test models chosen and/or adverse effects observed in animal studies.

The workshop participants also concluded that AOPs may be especially of use in the hazard identification of a food additive identifying the relevance of the target organ/system, model organism or type of adverse effect for humans. Furthermore, the workshop participants acknowledged that the use of AOPs can contribute to reducing and refining animal studies in the hazard identification of food additives. Indeed, the use of AOPs could facilitate the shift to a new paradigm of safety assessment that makes use of a human-based mechanistic approach rather than relying on the prediction and/or extrapolation of animal toxicity data. More research is needed to understand how AOPs could assist in the definition of a dose–response curve in the process of hazard characterization, which is essential to establish health-based guidance values. This would require development of quantitative AOPs that describe concentration–response curves for the rate-limiting step for induction of the adverse effect, which may subsequently be translated into in vivo dose–response curves using PBK models.

The workshop also indicated how regulators are currently using the AOP framework or envision its use in the future, since AOPs share many conceptual similarities with modes-of-action. In the future, use of AOPs for read-across from data-rich compounds to data-poor compounds is an opportunity, although read-across is currently very scarcely implemented in the safety evaluation of food additives. Noteworthy, for food flavors, which represent another category of food additives, use of read-across is an essential part of the regulatory framework (Cohen et al. 2018; EFSA 2010), so that a future role for AOPs in this field may become an option.

The question on what shortcomings and major gaps in knowledge are hampering the application of AOPs in the safety assessment of food additives was answered by pointing to the lack of AOPs for certain endpoints, such as gastrointestinal tract irritation, as well as for a number of non-specific, late-onset and/or local effects, including body weight changes, for which no AOPs have been developed.

The question on what is needed to address these gaps suggested the role of clinicians, although, as outlined above, effects of food additives in the clinic may be absent or perhaps limited to allergies/intolerances, which is an endpoint thus far not used as the basis for health-based guidance values for food additives.

The workshop was unable to provide an answer to the question of how one would apply the AOP framework across all the different types of food safety assessments, from screening to regulation. Considering what information is needed in the safety assessment of food additives, the necessity to integrate AOPs with knowledge of the kinetics of test chemicals was noted. Thus, integrating PBK models into the AOP concept may be an essential way forward for comprehensive non animal-based risk assessments.

The final question on how risk assessment methodologies can be adapted to best use AOPs can be related to the fact that current risk assessment methodologies already consider modes-of-action. A wider role for AOPs in future risk assessment may probably best focus on providing support for read-across. If so, AOPs can play a role in providing mechanistic support for alternative testing strategies, including QIVIVE and read-across.

So far, the use of AOPs in risk assessment has been applied successfully for the endpoint of skin sensitization, which has led to its regulatory acceptance in the cosmetics sector. Although a number of challenges need to be tackled to be able to apply the AOP framework in other sectors, including the food industry, considerable research effort has been made in the last years in that direction. It appears clear that the full realization of the AOP potential will significantly impact the current risk assessment paradigm, leading eventually to a human-focused rather than animal-based approach.

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Compliance with ethical standards

Conflict of interest This work was conducted by an expert group of the European branch of ILSI. Industry members of this task force are listed on the ILSI Europe website at https://www.ilsi.eu. For further information about ILSI Europe, please e-mail info@ilsieurope.be. The opinions expressed and the conclusions of this publication are those of the authors and do not necessarily represent the views of ILSI Europe nor those of its member companies. Experts are not paid for the time spent on this work. However, the non-industry members within the expert group were offered support for travel and accommodation costs from the "New approaches to chemical risk assessment for foods and food ingredients" task force to attend meetings to discuss the manuscript and a small compensatory sum with the option to decline. The research reported is the result of a scientific evaluation in line with ILSI Europe's framework to provide a precompetitive setting for public–private partnership. ILSI Europe facilitated scientific meetings and coordinated the overall project management and administrative tasks relating to the completion of this work. For further information about ILSI Europe, please email info@ilsieurope.be or call + 3227710014.

References

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL (2010) Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29:730–741. https://doi.org/10.1002/etc.34

Bal-Price A, Meek ME (2017) Adverse outcome pathways: application to enhance mechanistic understanding of neurotoxicity. Pharmacol Ther 179:84–95. https://doi.org/10.1016/j.pharmthera.2017.05.006

Battistoni M, Di Renzo F, Menegola E, Bois FY (2019) Quantitative AOP based teratogenicity prediction for mixtures of azole fungicides. Comput Toxicol 11:72–81. https://doi.org/10.1016/j.comtox.2019.03.004

Boobis AR, Cohen SM, Dellarco VL, Fenner-Crisp PA, Moretto A, Pastoor TP, Schoeny RS, Seed JG, Wolf DC (2016) Classification schemes for carcinogenicity based on hazard-identification have become outmoded and serve neither science nor society. Regul Toxicol Pharmacol 82:158–166. https://doi.org/10.1016/j.yrtph.2016.10.014

Cohen SM, Eisenbrand G, Fukushima S, Gooderham NJ, Guengerich FP, Hecht SS, Rietjens IMCM, Davidsen JM, Harman CL, Taylor SV (2018) Updated procedure for the safety evaluation of natural flavor complexes used as ingredients in food. Food Chem Toxicol 113:171–178. https://doi.org/10.1016/j.fct.2018.01.021

Corton JC, Peters JM, Klaunig JE (2018) The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. Arch Toxicol 92:83–119. https://doi.org/10.1007/s00204-017-2094-7

Dent M, Teixeira Amaral R, Amores Da Silva P, Ansell J, Boisleve F, Hatao M, Hirose A, Kasai Y, Kem P, Kreiling R, Milstein S, Montemayor B, Oliveira J, Richarz A, Taalman R, Vaillancourt R, Verma R, Posada NVORC, Weiss C, Kojima H (2018) Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients. Comput Toxicol 7:20–26. https://doi.org/10.1016/j.comtox.2018.06.001

Dent MP, Li H, Carmichael PL, Martin FL (2019) Employing dietary comparators to perform risk assessments for anti-androgens without using animal data. Toxicol Sci 167:375–384. https://doi.org/10.1093/toxsci/kfy245

Desprez B, Dent M, Keller D, Klaric M, Ouédraogo G, Cubberley R, Duplan H, Eilstein J, Ellison C, Grégoire S, Hewitt NJ, Jacques-Jamin C, Lange D, Roe A, Rothe H, Blaauboer BJ, Schepky A, Mahony C (2018) A strategy for systemic toxicity assessment based on non-animal approaches: the Cosmetics Europe Long Range Science Strategy programme. Toxicol In Vitro 50:137–146. https://doi.org/10.1016/j.tiv.2018.02.017

Doi AM, Hill G, Seely J, Hailey JR, Kissling G, Bucher JR (2007) α2u-Globulin nephropathy and renal tumors in National Toxicology Program studies. Toxicol Pathol 35:533–540. https://doi.org/10.1080/01926230701338941

EFSA (2005) Opinion of the Scientific Committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA J 282:1–31. https://doi.org/10.2903/j.efsa.2005.282

EFSA Panel on Food Contact materials, Enzymes, Flavourings and Processing Aids (2010) Guidance on the data required for the risk assessment of flavourings to be used in or on foods. EFSA J 8:1623. https://doi.org/10.2903/j.efsa.2010.1623

Erhlich VA, Dellafiora L, Mollergues J, Dall'Astra C, Serrant P, Marin-Kuan M, Lo Piparo E, Schilter B, Cozzini P (2015) Hazard assessment through hybrid in vitro/in silico approach: the case of zearalenone. Altex 32:275–286. https://doi.org/10.14573/altex.1412232

EU (2009) Regulation (EC) no. 1223/2009 of the European parliament and of the council of 30 November 2009 on cosmetic products (recast). Offi J Eur Union L342:59–209

Hill AB (1965) The environment and disease: association or causation? Proc R Soc Med 58:295–300. https://doi.org/10.1177/014107681456271 8. https://www.epa.gov/chemical-research/toxicity-forecasting **(consulted January 2020)**

Jeong J, Choi J (2017) Use of adverse outcome pathways in chemical toxicity testing: potential advantages and limitations. Environ Health Toxicol 33:e2018002. https://doi.org/10.5620/eht.e2018002

Kang DS, Yang JH, Kim HS, Koo BK, Lee CM, Ahn YS, Jung JH, Seo YR (2018) Application of the adverse outcome pathway framework to risk assessment for predicting carcinogenicity of chemicals. J Cancer Prev 23:126–133. https://doi.org/10.15430/JCP.2018.23.3.126

Kramer NI, Hoffmans Y, Wu S, Thiel A, Thatcher N, Allen TEH, Levorato S, Traussnig H, Schulte S, Boobis A, Rietjens IMCM, Vinken M (2019) Characterizing the coverage of critical effects relevant in the safety evaluation of food additives by AOPs. Arch Toxicol 93:2115–2125. https://doi.org/10.1007/s00204-019-02501-x

Levorato S, Rietjens IMCM, Carmichael PL, Hepburn PA (2019) Novel approaches to derive points of departure for food chemical risk assessment. Curr Opin Food Sci 27:139–144. https://doi.org/10.1016/j.cofs.2019.02.016

Louisse L, Beekmann K, Rietjens IMCM (2017) Use of physiologically based kinetic modeling-based reverse dosimetry to predict in vivo toxicity from in vitro data. Chem Res Toxicol 30:114–125. https://doi.org/10.1021/acs.chemrestox.6b00302

Nepelska M, Odum J, Munn S (2017) Adverse outcome pathway: peroxisome proliferator-activated receptor α activation and reproductive toxicity: development and application in assessment of endocrine disruptors/reproductive toxicants. Appl In Vitro Toxicol 3:234–249. https://doi.org/10.1089/aivt.2017.0004

Perkins EJ, Ashauer R, Burgoon L, Conolly R, Landesmann B, Mackay C, Murphy CA, Pollesch N, Wheeler JR, Zupanic A, Scholz S (2019) Building and applying quantitative adverse outcome pathway models for chemical hazard and risk assessment. Environ Toxicol Chem 38:1850–1865. https://doi.org/10.1002/etc.4505

Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen, London

Thomas RS, Bahadori T, Buckley TJ, Cowden J, Deisenroth C, Dionisio KL, Frithsen JB, Grulke CM, Gwinn MR, Harrill JA, Higuchi M, Houck KA, Hughes MF, Hunter ES, Isaacs KK, Judson RS, Knudsen TB, Lambert JC, Linnenbrink M, Martin TM, Newton SR, Padilla S, Patlewicz G, Paul-Friedman K, Phillips KA, Richard AM, Sams R, Shafer TJ, Setzer RW, Shah I, Simmons JE, Simmons SO, Singh A, Sobus JR, Strynar M, Swank A, Tornero-Valez R, Ulrich EM, Villeneuve DL, Wambaugh JF, Wetmore BA, Williams AJ (2019) The next generation blueprint of computational toxicology at the US Environmental Protection Agency. Toxicol Sci 169:317–332. https://doi.org/10.1093/toxsci/kfz058

Villeneuve D, Volz DC, Embry MR, Ankley GT, Belanger SE, Léonard M, Schirmer K, Tanguay R, Truong L, Wehmas L (2014) Investigating alternatives to the fish early-life stage test: a strategy for discovering and annotating adverse outcome pathways for early fish development. Environ Toxicol Chem 33:158–169. https://doi.org/10.1002/etc.2403

Wetmore BA, Wambaugh JF, Allen B, Ferguson SS, Sochaski MA, Setzer RW, Houck KA, Strope C, Cantwell K, Judson RS, LeCluyse E (2015) Incorporating high-throughput exposure predictions with dosimetry-adjusted in vitro bioactivity to inform chemical toxicity

testing. Toxicol Sci 148:121–136. https://doi.org/10.1093/toxsci/kfv171

Zgheib E, Gao W, Limonciel A, Aladjov H, Yang H, Tebby C, Gayraud G, Jennings P, Sachana M, Beltman JB, Bois FY (2019) Application of three approaches for quantitative AOP development to renal toxicity. Comput Toxicol 11:1–13. https://doi.org/10.1016/j.comtox.2019.02.001