Short Communication

Bridging the Gap Between Regulatory Acceptance and Industry Use of Non-Animal Methods

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Summary

Collaboration between industry and regulators resulted in the development of a decision tree approach using *in vitro* or *ex vivo* assays to replace animal tests when determining the eye irritation potential of antimicrobial cleaning products (AMCPs) under the United States Environmental Protection Agency (EPA) Office of Pesticide Programs' hazard classification and labeling system. A policy document issued by the EPA in 2013 and updated in 2015 describes the alternate testing framework that industry could apply to new registrations of AMCPs and, on a case-by-case basis, to conventional pesticide products. Despite the collaborative effort, the availability of relevant non-animal methods, and the EPA's change in policy, only a limited number of AMCPs have been registered using the framework. Companies continue to conduct animal tests when registering AMCPs due to various challenges surrounding adoption of the new testing framework; however, recent discussions between industry, regulators, and other interested parties have identified ways these challenges may be overcome. In this article we explore how use of the alternate framework could be expanded through efforts such as increasing international harmonization, more proactively publicizing the framework, and enhancing the training of regulatory reviewers. Not only can these strategies help to increase use of the EPA alternate eye irritation framework, they can also be applied to facilitate the uptake of other alternative approaches to animal testing in the future.

Keywords: non-animal testing strategy, eye hazard classification, EPA, antimicrobial cleaning products, pesticides

1 Introduction

In the United States, the majority of household cleaning products do not have to go through a registration process before they are marketed. Such products are under the jurisdiction of the Consumer Products Safety Commission, and the individual product manufacturers have the responsibility to decide how to assess safety, which can be done without using animals. However, if the product has an "antimicrobial" claim, it is considered a pesticide and is regulated by the Environmental Protection Agency's (EPA's) Office of Pesticide Programs (OPP). Historically, the EPA required animal testing, including eye irritation testing in rabbits, to determine the hazard classification of antimicrobial cleaning products (AMCPs).

Discussions on developing a non-animal approach (one that does not use live animals) to eye irritation testing began at the fall 2003 meeting of the Pesticide Program Dialog Committee (PPDC), an EPA federal advisory committee representing the pesticide industry, growers, the environmental and animal welfare communities, and other stakeholders. This led to collaboration between the EPA, seven AMCP companies, the Institute for In Vitro Sciences (IIVS), and the Accord Group in 2004. The companies provided eye irritation data from both historic *in vivo* testing (no new animal testing was conducted) and *in vitro* and *ex vivo* methods, which allowed for a retrospective comparative analysis in addition to targeted prospective *in vitro* and *ex vivo* testing and analysis (ICCVAM, 2010). The non-animal methods used were: the bovine corneal opacity and permeability (BCOP) assay (Gautheron et al., 1994), the Cytosensor™ microphysiometer (CM) assay (Hartung et al., 2010), and the EpiOcular™ (EO) time to toxicity assay (MatTek Corporation, Ashland, MA) (Stern et al., 1998). The protocols, prediction models for EPA labeling categories, applicability domains, and suggested integrated use of these assays were defined specifically for this program and are described in the EPA policy document (U.S. EPA OPP, 2015 (revised)).

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Tab. 1: Signal words and hazard statements from U.S. EPA OPP eye hazard categories I through IV
Category I substances are corrosives, category II substances cause substantial but temporary eye injury, category III substances are moderate eye irritants, and category IV substances are non-irritants. A major difference between categories is the personal protective equipment that must be worn and described on the label (from U.S. EPA OPP 2015 (revised)).

Toxicity Category	Signal Word	Eye Protection and Label Precautionary Language
I	DANGER	Goggles face shield, or safety glasses. Corrosive. Causes irreversible eye damage.
II	WARNING	Goggles face shield, or safety glasses. Causes substantial but temporary eye injury.
III	CAUTION	Protective eyewear if appropriate. Causes moderate irritation.
IV	CAUTION	No statement required.

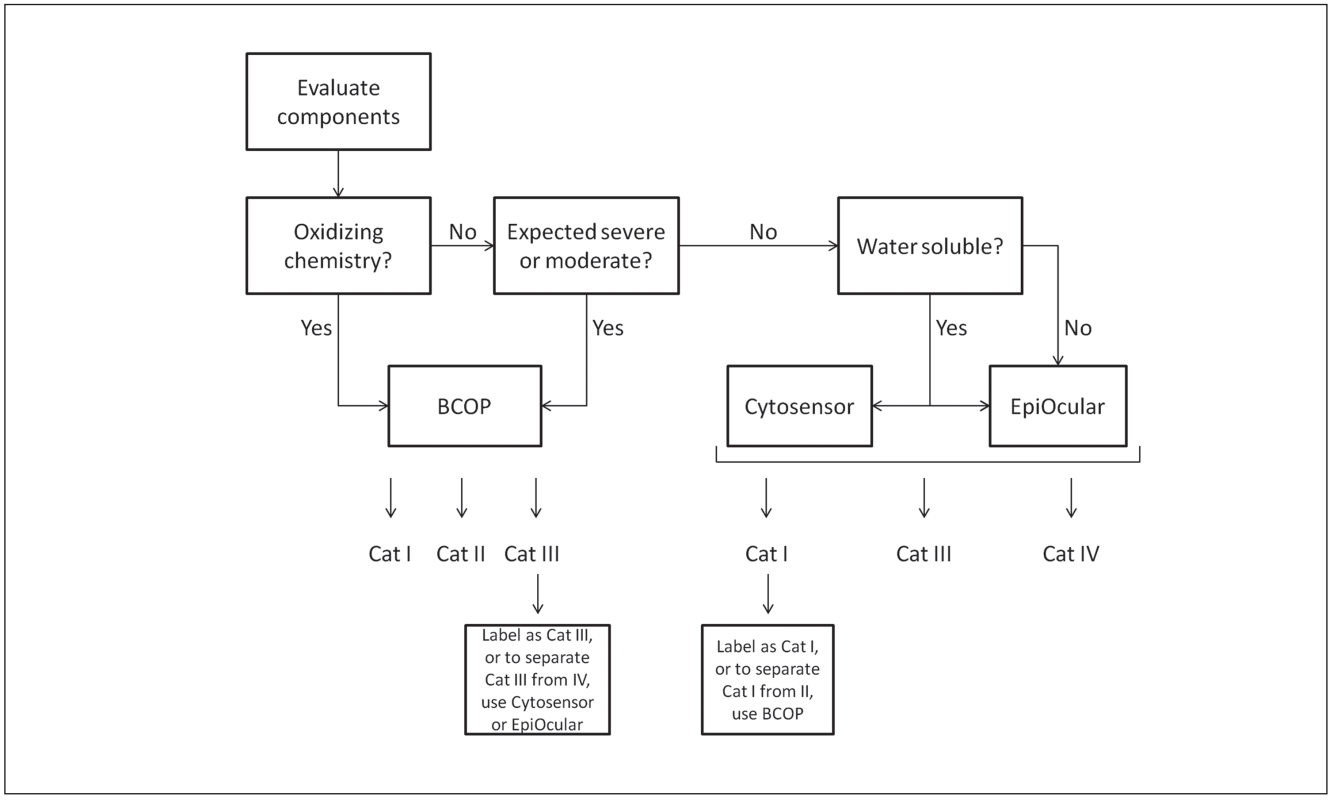

Fig. 1: The EPA framework shows how to use the assays in a decision approach
The appropriate assay is selected by evaluating the test substance. If it has oxidizing chemistry or is expected to be a severe or moderate irritant, the BCOP assay can be used for determination of U.S. EPA toxicity categories I, II, and III for eye irritation. If a test substance is identified as category III by BCOP, the manufacturer can choose to either label the product as category III or to conduct the Cytosensor™ or EpiOcular™ assays to differentiate between categories III and IV. Alternatively, if the test substance is expected to be mild or non-irritating, the Cytosensor™ or EpiOcular™ assays can be used for determination of U.S. EPA toxicity categories I, III, and IV for eye irritation. If the test substance is identified as category I by the Cytosensor™ or EpiOcular™ assays, the manufacturer can choose to either label the product as category I or to conduct the BCOP to distinguish between categories I and II. Thus, while one assay is not sufficient to classify all four EPA eye irritation categories, it is possible to conduct only one assay, assuming some knowledge of the test substance (Modified from U.S. EPA OPP 2015 (revised)).

The promising results prompted the EPA to initiate an 18-month pilot program in 2009 during which companies were encouraged to submit eye irritation studies on AMCPs using the proposed *in vitro* methods and testing strategy. The purpose of this pilot program was to determine the effectiveness of the alternate approach in classifying AMCPs into one of the EPA's four categories for eye hazard labeling (Tab. 1). After analysis of the pilot program submissions, the EPA issued a policy in 2013 that allowed for the use of this alternate framework with AMCPs and, on a case-by-case basis, with conventional pesticide products (U.S. EPA OPP, 2015 (revised)). The effort was successful in demonstrating a cooperative approach between the EPA and stakeholders in developing an alternate testing framework for measuring the eye irritation potential of AMCPs without the use of live animals (Fig.1) (Curren, 2014). It also demonstrated that methods that achieved the dual goals of promoting animal welfare and meeting the rigors of scientific and regulatory standards could be successfully applied.

2 Results

While enactment of the EPA policy was the result of significant cooperation and collaboration amongst the parties involved, according to the EPA, only 10 AMCPs (< 5% of registered AMCPs) have been registered using the alternate framework since 2009 (personal communication, EPA OPP). In an effort to understand why there is such underutilization of this program, IIVS and People for the Ethical Treatment of Animals (PETA) sent a questionnaire to eleven major AMCP companies, including those that had participated in the collaborative effort, asking if they were using the animal test or were following the available alternate framework, and why. We also asked if any of their non-animal submittals had been successful, and if not, what contributed to the lack of acceptance by the EPA. In addition, we asked for suggestions for how the policy could be improved and to identify any countries that do not accept non-animal methods. While only five companies responded, the replies as to why the alternate framework was not being widely used were fairly consistent and can be summarized as follows:

- Lack of global regulatory acceptance (meaning that the animal test would likely be conducted anyway since it was required by other countries or certain U.S. state authorities)
- Uncertainty within industry about regulatory reviewers' familiarity with the framework and their ability to evaluate and interpret non-animal studies, which could influence the likelihood of acceptance
- Concern that the alternate testing strategy might overestimate the hazard of the product
- Time and cost involved if a company had to do both the non-animal test(s) and the animal test
- Time and cost to a company if the regulatory agency took longer to register the product due to unfamiliarity with the non-animal methods

3 Discussion

The continued underutilization by industry of the alternate testing framework, which required substantial resources from the EPA to implement, could result in a vicious cycle whereby the agency would be hesitant to put further resources into the program. Without commitment from industry, the EPA may become reluctant to take additional steps such as offering incentives (e.g., faster reviews) to companies that submit non-animal test data, or expanding the training of reviewers in evaluating this data. This could lead to even less use by industry. A failed registration due to use of non-animal methods would likely discourage a company from trying again, as well as deter others that have not yet tried to submit non-animal data. Early difficulties with a novel testing program can be extremely detrimental to its success.

This illustrates the importance of leadership, both within industry and at the EPA. Overcoming institutional inertia at companies and regulatory agencies requires collaboration among a motivated group of people across multiple sectors. Success depends on companies and regulators taking some risks and being willing to lead the charge towards the new testing paradigm. By reducing the use of animals in safety testing, companies can benefit in the long term by marketing their products as such, but they must be willing in the short term to troubleshoot the new system and face potential delayed regulatory acceptance as the EPA becomes proficient in reviewing non-animal test data. In order to incentivize industry, the EPA must commit to quickly educating their reviewers on new testing paradigms to minimize any delay in reviews, and/or provide for expedited review relative to submissions with animal data only.

Although industry's limited use of the alternate eye irritation classification strategy has been disappointing thus far, the development of the policy is an attestation of the ability of industry, a regulatory agency, and non-governmental organizations (NGOs) to address a common goal. Furthermore, most of industry's concerns can be resolved, or at least ameliorated, if action is taken on the issues described below.

3.1 Full regulatory acceptance

Full regulatory acceptance – that is, among U.S. agencies and internationally – is critical to the uptake of new methods. Companies cite a lack of full regulatory acceptance as a major barrier to the use of the eye irritation alternate testing framework. This lack of harmonization applies to issues with global acceptance as well as uncertainty about acceptance by U.S. states such as California, which perform their own pesticide reviews in addition to federal review. Companies are reluctant or unwilling to spend the time and money conducting the non-animal test if the animal test will still be required by other regulatory agencies. For example, depending on the type of products, regulators in Russia, China, Brazil, Turkey and many other countries still require animal data for acute ocular and dermal toxicity studies. However, some companies are also conducting non-animal tests in situations where *in vivo* testing is required because paired data of this type promotes further acceptance of the non-

animal methods. Armed with parallel sets of *in vitro* / *ex vivo* and *in vivo* test data and the knowledge that the EPA accepts the non-animal methods, these companies will have a greater ability to push for acceptance of these methods in other countries. Likewise, with its policy in place, EPA regulators now have the opportunity to more vigorously engage in discussions with their counterparts at international forums, such as International Cooperation on Alternative Test Methods (ICATM) meetings, to foster global harmonization.

3.2 Reviewer awareness and proficiency with non-animal methods

While the EPA has issued a clear policy stating that it will accept non-animal methods for classifying eye hazard, depending on a reviewer's familiarity with the policy and the methods, there may be inconsistent acceptance of the submitted studies among reviewers. Some companies have told us that their registration submittals have been met with the response that the *in vitro* or *ex vivo* methods are not accepted by EPA. These are clearly situations where the reviewer was not familiar with the EPA OPP's acceptance of the alternate testing framework for AMCPs. Lack of awareness of existing approved non-animal testing strategies by some reviewers leaves industry less willing to submit these methods.

Reviewer unfamiliarity with the policy and methods also has the potential to lead to longer review times. Regulatory agencies need to proactively prepare for incoming registration submissions, even before many are received, so that companies' first experiences with the new process go as smoothly as possible. Slow regulatory review will lead to fewer submissions by companies that want to avoid delays in their products reaching the market. To counter this delay and to incentivize companies, regulatory agencies could offer expedited review of submissions that use non-animal methods.

Regular training of reviewers will facilitate proficiency with evaluating studies using new methods and decrease the likelihood of delayed reviews and rejections. The EPA has shown its commitment to the process by holding, in collaboration with IIVS, an in-person training session on the AMCP non-animal testing strategy (and other non-animal methods) for its staff in August 2015, and has stated that they intend to continue with such activities (personal communication, EPA OPP). To be effective, training must take a variety of forms, and include repeat training sessions, training of all new reviewers, and constant availability of updated information (e.g., factsheets and videos). It will also require participation from the EPA, industry, and NGOs to develop and promote training workshops and materials. Training must reach across all sectors and levels of management within an organization. Even with a training program in place, some challenges with reviewer variability will likely remain, and companies should be persistent if it appears non-animal test data were rejected in error.

3.3 Accurate prediction of hazard categories

Another barrier noted by industry is the possibility of over-prediction of hazard category by the non-animal tests. While it is known and accepted by regulators and industry that non-animal eye irritation tests may predict slightly higher or lower than the *in vivo* Draize test, companies are not comfortable with what they consider the level of over-prediction seen in some cases. The more irritating a substance is predicted to be, the more warnings and requirements for protective personal equipment must be included on the label. Therefore, it becomes a concern for consumers and likewise companies if a non-animal method consistently predicts a more hazardous category for the product than the animal test might have.

A key factor to recognize when defining acceptable levels of false negatives and false positives for any non-animal approach is the variability in animal test data (Bruner et al., 2002). The results from animal tests are often reported as a single data point with no error bars; however, the error bars may actually extend over multiple hazard categories. Repeating the animal test may produce results that also over-predict the previous results, as the non-animal tests sometimes do. Because variability exists with any test, industry and regulators should work to reach agreement regarding the level of over-prediction as well as under-prediction that is acceptable in the non-animal tests compared to the animal test. In this case, industry and the EPA could discuss whether it might make sense to modify the prediction algorithm of the non-animal tests. It should also be noted that the regulatory animal test cut-off values for each hazard category were subjectively developed and it may be advantageous to discuss modification of the category range values to satisfy industry concerns without any adverse effect on safety.

3.4 Outreach to industry

Due to the inevitable questions that arise during a new process, it is critical that an ongoing dialog be maintained within the EPA and with industry to discuss obstacles and experiences. Since smaller companies are more likely to lack knowledge of new policies, the EPA could take a proactive approach in reaching out to them, including contacting any registrant whose products could now be registered using the alternate framework. The EPA could also recommend or require the use of non-animal tests in future submissions; however, requiring use of non-animal tests would involve promulgation of new regulations by the EPA or inclusion of language in federal law that prohibits animal use if non-animal alternatives are available. At a minimum, registrants who submit *in vivo* eye irritation data could be required to concurrently submit non-animal test data following the alternate testing framework guidelines. Another concern for smaller companies could be a lack of in-house expertise to design studies and interpret the results from non-animal methods. As demand for non-animal methods increases, contract laboratories may see profitability in offering services in this area to smaller companies to fill this gap. A collaborative training effort between the EPA, industry, and NGOs could also help address this issue. In addition, states that perform their own pesticide reviews could facilitate efforts by issuing policy decisions to accept the non-animal method data and adding information about the program to their websites, as the federal agency has done.

3.5 Monitoring success

The EPA should enhance its database system to allow for easy monitoring of the use of new methods. It is important to track how often data from the non-animal methods are submitted by industry, whether the data are accepted or rejected by reviewers, and the reasons for rejection. This monitoring will inform as to whether companies are implementing the methods or, if not, will provide a basis for stakeholders to work together to identify and address any remaining barriers to use. In part due to the current lack of a monitoring system, it is only recently that stakeholders have become aware of the low number of non-animal eye studies that have been accepted since the enactment of the policy. This clearly shows the urgent need to reinstate discussions on how to improve use of the framework.

3.6 Beyond eye irritation

The EPA has demonstrated commitment to implementing non-animal methods by establishing a policy on the use of an alternate testing framework for classification of eye irritation potential of pesticide products. It has also set near term goals for reducing and replacing the use of animals for other acute endpoints, including skin irritation, skin sensitization, and acute dermal toxicity (U.S. EPA OPP, 2016a). In fact, some progress has already been made in performing the activities we recommend above. Meetings were held in October 2015 and January 2016 between the EPA, AMCP and conventional pesticide companies, NGOs, industry trade associations, state representatives, and internal staff to identify and discuss barriers to the use of non-animal approaches for acute toxicity testing and what could be done to overcome these barriers. Outcomes included the EPA expressing in an open letter to stakeholders (U.S. EPA OPP, 2016b) its willingness to lead the adoption of non-animal methods for pesticides, with the anticipation that other regulatory agencies will follow. It also agreed to facilitate project management but noted that strong participation and support is needed from industry and other stakeholders.

Efforts are currently underway by the EPA and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to collect, curate, and analyze existing sets of parallel *in vivo* and *in vitro* / *ex vivo* skin irritation and skin sensitization test data for conventional pesticides personal communication, NICEATM staff and U.S. EPA OPP (2016d), which could lead to optimization of prediction algorithms. EPA-stakeholder work groups are being formed to assist on these projects (U.S. EPA OPP, 2016b).

In other areas related to reducing animal use, the EPA released draft guidance for waiving acute dermal testing for pesticide formulations based on a retrospective analysis that shows acute oral data are sufficiently predictive of dermal results (U.S. EPA OPP, 2016c). The EPA also released final guidance for a stepwise process for establishing and implementing alternative approaches to *in vivo* studies that can be applied to any of the acute toxicity tests (U.S. EPA OPP, 2016d).

3.7 Transition to GHS

For some acute toxicity effects the EPA and GHS systems have the same number of hazard categories but for others they differ, and the category cutoff ranges often vary between the two systems (U.S. EPA OPP, 2004). Rather than developing new algorithms for *in vitro* tests to predict EPA hazard categories, a less complicated approach would be for the EPA to transition from its current system of hazard classification to the GHS one for which non-animal tests have already been validated as described in OECD test guidelines (e.g., OECD, 2012, 2013, 2015). The EPA is giving serious consideration to adopting the GHS classification and labeling system (U.S. EPA OPP, 2016b), but indicated at stakeholder meetings that such a transition would take time and require several steps. For companies that sell products globally, testing to meet the requirements of only one classification and labeling system would undoubtedly save time and reduce costs.

4 Conclusions

The implementation of new test methods is a multi-step process that does not end with the enactment of a policy. The process progresses from planning through data collection and analysis, to policy development, public commenting, outreach, and education of regulatory and industry personnel, and finally to the widespread use and acceptance of the new methods by industry and regulators. The EPA alternate testing framework for classifying eye irritation potential has achieved most of these milestones, but to see significant reductions in animal use, industry, the EPA, and global agencies must do more to increase the use and acceptance of the non-animal methods.

As new methods and testing strategies are developed and approved for other toxicity endpoints, this multi-step process will need to be repeated. Successful implementation will require companies and regulatory agencies to display fortitude and leadership in heralding in the new toxicity testing paradigm based on human-relevant mechanisms. This will lead to the common goal of better protection of human health and replacement of animals in testing.

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Conflict of interest

The authors state they have no conflicts of interest.

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