

# Editorial

## The EU Commission's *Draft Report on Alternative (Non-animal) Methods for Cosmetics Testing: Current Status and Future Prospects — 2010: A Missed Opportunity*

On 23 July 2010, the European Commission initiated a public consultation on the *Draft Report on Alternative (Non-animal) Methods for Cosmetics Testing: Current Status and Future Prospects — 2010*.<sup>1</sup> The draft report was prepared by five groups of experts, nominated by stakeholders, who were asked to provide a broad and objective picture of the scientific and technical issues related to establishing alternative test methods for the five human health(-related) effects falling under the 2013 deadline for the marketing ban of the EU Cosmetics Directive. It was also intended that the draft report would contain, where possible, a science-based estimate of the time necessary to achieve full replacement of animal testing for the respective endpoints.

The background to this consultation is the 7th Amendment to the EU Cosmetics Directive, *Directive 76/768/EEC*, which calls for a marketing ban, from 11 March 2013, on cosmetic products which contain ingredients tested in animals for repeated dose toxicity (including skin sensitisation and carcinogenicity), reproductive toxicity and toxicokinetics.<sup>2</sup> The draft report consists of five individual chapters, each of which addresses one of the specific human health(-related) effects. In 2011, the Commission will have to inform the European Parliament and the Council about the status that non-animal methods will be expected to have reached by 2013.

This activity is, of course, very good news for the general public in Europe and for the animal welfare movement, who are looking forward to the day on which animals will no longer have to be sacrificed for cosmetics testing. However, a close look at the five draft chapters of the report shows that an important opportunity to come up with new ideas for the way forward has been missed, as is outlined in the *Comment* by Michael Balls and Richard Clothier, from FRAME, in this issue of *ATLA*.<sup>3</sup> This is most unfortunate, since the authors of the individual chapters had a chance to provide an inventory of alternative methods that are, or would soon be, ready for use, and to offer a realistic view on how to implement these methods in the regulatory testing of cosmetic ingredients. In contrast, the five draft reports are inventories of current *in vivo* animal methods, usually OECD Test Guide-

lines (TGs), and *in vitro* methods that are available today, with some mention of methods that show some promise of reaching readiness for prevalidation, as defined by ECVAM. However, there are no specific proposals on how the new non-animal methods could be integrated into a specific testing strategy for cosmetics ingredients.

In fact, a general chapter is missing, which could have summarised the current status of the non-animal methods that are described in the five draft chapters on testing for individual human health effects, and how to integrate the individual tests in a testing strategy covering all the five health effects. This is not acceptable, from both the scientific perspective and the animal welfare perspective, since guidance has been provided in opinions published by the Scientific Committee on Consumer Safety (SCCS, formerly the Scientific Committee on Consumer Products [SCCP]) in recent years,<sup>4</sup> which indicates that, when considerable oral intake is expected, or when dermal penetration data suggest a significant systemic absorption, information on toxicokinetics, carcinogenicity and reproductive toxicity “may become necessary”. Since no more-detailed information has been provided by the SCCS to date, one would expect that the five expert groups would have come up with proposals on the minimum testing requirements *in vivo*, and how to develop and use non-animal methods to replace them, or how to combine *in vivo* methods and non-animal methods in integrated testing strategies for the five human health-related endpoints.

Since I have worked for the past 40 years in reproductive toxicology, in academia and as a regulator, with experience in the use of both *in vivo* and *in vitro* methods, I want to focus my remarks on draft Chapter 5, on reproductive toxicity. In addition, my book on drug treatment in pregnancy and lactation<sup>5</sup> (in German) is in its 7th edition. It is the standard text on this topic for obstetricians and paediatricians in Germany, Austria and Switzerland, and it has recently been translated into English and Russian.

In an editorial in *ATLA* in 1986 on REACH testing requirements,<sup>6</sup> I summarised the testing regulations in the area of reproductive toxicity. The complexity of the reproductive system and the vast

number of tissue targets for the induction of malformations or post-natal effects, provide the rationale for the toxicity testing of chemicals in highly-standardised and internationally harmonised animal tests. For historical reasons, the spectrum of reproductive toxicity testing is markedly different for drugs and for chemicals. In preclinical testing during drug development, “segment 1, 2 and 3” studies have to be conducted, which cover pregnancy as well as pre-natal and post-natal toxicity, and also the lactation period. In contrast, the regulatory testing requirements for industrial chemicals mainly depend on the production volume of the chemical. These requirements include two types of reproductive toxicity test: a developmental toxicity study, and one-generation or two-generation “fertility” studies. Details are given in the figures and tables of my recent review, *The way forward in reproductive/developmental toxicity testing*.<sup>7</sup>

The implementation of the new EU REACH legislation<sup>8</sup> is expected to result in a dramatic increase in the number of toxicological studies that rely on animal experimentation.<sup>9</sup> For both economical and ethical reasons, there is a great demand for new non-animal tests in the field of reproductive toxicology, since they can usually be conducted much faster than the animal tests, and are usually much less expensive to perform. From a Three Rs perspective, it is legitimate to ask why the current reproductive toxicity test requirements for industrial chemicals and pesticides are so different from those for drugs. It is a major challenge to discover why the safety testing approach to reproductive toxicity, which is used for drugs and which has proven effective for the past 50 years, cannot also be used for other chemicals, especially since drugs are produced to be taken into the human body, while industrial chemicals and pesticides are produced and used for other purposes, with the aim of reducing human exposure as much as possible.<sup>7</sup>

This basic, and obvious, question about regulatory testing for reproductive toxicity has not been taken into account by the authors of draft Chapter 5. They did not even consider the recent OECD *Guidance Document 43 on Mammalian Reproductive Toxicity Testing and Assessment*.<sup>10</sup> In essence, before giving an inventory of the currently available *in vivo* and non-animal methods in reproductive toxicology, the experts should have critically defined the specific information requirements for the safety assessment of cosmetics. However, they have merely reported on the current situation, according to which, “*When considerable oral intake is expected, or when dermal penetration data suggest a significant systemic absorption, information on... reproductive toxicity “may become necessary”. Additional recommendations on specific in vivo or in vitro reproductive toxicity studies to be submitted with*

*a dossier are not described in the Notes on Guidance. From the SCCS/SCCP opinions published within recent years (2000–2009) it can be concluded that in most cases an in vivo developmental toxicity study in the rat (OECD TG 414) — submitted by the manufacturer as the only study on reproductive toxicity — was considered sufficient by the SCCS.”* It is important to note that, according to the introductory document to the public consultation, the Commission expects “*justified comments on the conclusions*”.

This aspect has not been considered in the draft chapter on reproductive toxicity. If the *in vivo* developmental toxicity study in the rat (OECD TG 414) is the basis of the information commonly provided in dossiers, and the SCCS accepts this information for regulatory purposes, it is not sufficient to provide an inventory of all the *in vivo* and *in vitro* tests that are currently available for reproductive toxicity testing. There are two tasks that the experts would have been expected to have addressed:

1. The circumstances that would require the additional testing of cosmetic ingredients in a one-generation or a two-generation study, since these are the only *in vivo* tests that are accepted according to the REACH legislation.
2. The validation status of currently available *in vitro* tests for developmental toxicity (OECD TG 414).

This approach would have been straightforward, and it takes into account the fact that developmental toxicity is the most important irreversible endpoint in reproductive toxicity.

For reasons that are not explained in the draft chapter, the experts did not consider this basic concept of exposure-driven toxicity testing, and, in addition, up to now, the SCCS has not published any guidance on this issue. Thus, although there is specific legislation in place in Europe for the safety testing of cosmetics, a specific and exposure-driven testing concept still has to be devised and implemented. Bearing in mind that, even for the *in vivo* testing of drugs, we do not need one-generation or two-generation studies to cover the reproductive cycle, the experts of working group 5 should have developed a realistic proposal that would not rely on one-generation or two-generation studies. No explanation is given as to why *in vivo* tests such as the screening assays (OECD TGs 421 and 422), and the developmental neurotoxicity test (OECD TG 426), are described in detail, or why the Uterotropic Bioassay, the Hershberger Bioassay, and the Transfected Human Oestrogen Receptor- $\alpha$  Transcription Activation Assay (OECD TGs 440, 441, 455) are described for the testing of cosmetic

ingredients. So far, they have not been used for cosmetics testing as reported by the SCCS, and no reason is given as to why these *in vivo* assays are considered in the draft chapter.

Rather than using the most obvious testing approach, the experts have chosen the complex reproductive cycle as their starting point, and they repeatedly state, throughout the report, that it may take decades before we will be able to set up an integrated testing strategy that covers the whole reproductive cycle. As a consequence, only the first and last three pages of draft Chapter 5 are devoted to the task that the experts should have set out to accomplish. Instead, they have provided an inventory of current OECD *in vivo* TGs for reproductive toxicity (seven pages), and on the following 14 pages, an inventory of all the *in vitro* tests that have ever been developed in the field of reproductive toxicity, including tests for endocrine disruption, irrespective of the status of their development and/or prevalidation/validation. *Table 1* occupies six pages, and summarises the previous text on *in vivo* and *in vitro* methods. Only on the last three pages, which do not refer to the sections on *in vivo* and *in vitro* tests, is a vague perspective given on what may be achieved in this field of toxicology within the next decades. In essence, the report does not contain any “justified comments on the conclusions” for cosmetics testing, as requested by the Commission.

This general deficiency is, in particular, illustrated by *Table 1*, the “Inventory of available alternative methods”, in which no information is provided on how the validation status of the individual alternative tests was assessed, e.g. how a test for “Interaction with the aryl hydrocarbon receptor” can be used to provide information that is currently obtained in testing according to OECD TGs 414, 415 and 416. The last sentence of the legend to *Table 1* shows that the experts basically have no idea as how to approach the problem of developing a series of *in vitro* tests covering the reproductive cycle, since it reads: “*The list of alternative assays in this table comprises only a small proportion of the tests anticipated to be needed to replace in vivo reproductive toxicity testing covering the full reproductive cycle.*” There is no comment or suggestion on how to overcome this problem.

On the other hand, it is surprising that several of the draft chapter’s authors have recently participated in the successful “*ReProTect feasibility study, a novel comprehensive in vitro approach to detect reproductive toxicants*”,<sup>11</sup> and that they do not refer to their publication in draft Chapter 5. In this study, 10 blinded chemicals, with toxicologically well-documented profiles, were analysed by employing a test battery of 14 *in vitro* assays. The study’s authors reported that “*comparative*

*analysis together with a weight of evidence approach allowed a robust prediction of adverse effects on fertility and embryonic development of the 10 test chemicals in vivo.* In summary, the vast majority of the predictions made based on the *in vitro* results turned out to be correct when compared to the whole animal data. The procedure used here, a nearest neighbour analysis coupled with a weight of evidence approach, may guide future activities in the field of alternative toxicity testing.” From the viewpoint of the scientific community, from the animal welfare perspective, and, in particular, the expectations of the Health and Consumers Directorate-General (DG), as it considers the report it must make to the Parliament and the Council, it cannot be acceptable that the promising results of the ReProTect feasibility study, which was funded by the Commission’s Research DG, are not presented and discussed in draft Chapter 5.

There are several indications that the draft chapter seems to have been drafted under time constraints that did not allow proper editing. Since I do not want to waste time and space, I will just point to a few obvious mistakes. First of all, the reproductive cycle, which is shown in *Figure 1*, does not contain the central element of “maturation and production of gametes” (for details, see reference 7), which is a crucial step in pre-natal development, since male and female fertility depend on the undisturbed development of primordial germ cells. Again, from the scientific perspective, such a basic mistake is unacceptable.

Another weakness of the draft chapter concerns the coverage of alternative methods as far as the use of embryonic stem cells for developmental toxicity testing is concerned. Here, I must admit that I am biased, since the mouse embryonic stem cell test (mEST) was developed in my laboratory,<sup>12, 13</sup> and I successfully managed the ECVAM validation study on three *in vitro* embryotoxicity tests.<sup>14</sup> Since the mEST is conducted with a permanent mouse ES cell line, it has become more popular than other *in vitro* embryotoxicity tests, mainly because no pregnant animals have to be sacrificed. However, the prediction model of the mEST did not perform sufficiently well in the ReProTect study, when test chemicals were selected that were categorised according to a different classification system.<sup>15</sup> However, in the ReProTect feasibility study, the mEST provided correct classifications for the new test set of 10 chemicals. Moreover, the mEST has been established in several laboratories in the international drug industry, e.g. Hoffmann-La Roche, Switzerland,<sup>16</sup> Pfizer, USA,<sup>17</sup> and Sumitomo, Japan.<sup>18</sup> In addition, it was recently shown that a metabolic activation system employing human primary hepatocytes, can be used to expand the applicability of the mEST,<sup>19</sup> and that protein biomarkers can be used in the mEST to

identify distinct classes of toxic substances with clear pathway-related differences.<sup>20</sup> Most recently, it was demonstrated that human embryonic stem cells and metabolomics can be used successfully to predict the human developmental toxicity of pharmaceuticals.<sup>21</sup> Again, the authors of the draft chapter have failed to include these relevant publications, which are freely-accessible in the scientific literature, and have failed to provide an unbiased report on the use of embryonic stem cell-based assays for the *in vitro* developmental toxicity testing of cosmetic ingredients.

By contrast, however, the draft report provides detailed information on *in vitro* embryotoxicity tests which are outdated or have not been accepted to undergo formal validation, e.g. the amphibian embryo test (FETAX) and the Chicken Embryotoxicity Screening Test (CHEST). Again, the experts do not give any indication as to how these tests could be used for cosmetics safety testing.

These few examples clearly show that an excellent opportunity to promote the use of alternative non-animal methods for reproductive toxicity testing has been missed. The Health and Consumers DG of the Commission would be well advised to refuse to accept and publish the chapter on reproductive toxicity in its present form, or to use it in preparing the report it must make to the Parliament and the Council.

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