Toxicology in Vitro xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Toxicology in Vitro

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

Moving forward in carcinogenicity assessment: Report of an EURL ECVAM/ESTIV workshop

Raffaella Corvi[a,*], Federica Madia[a], Kathryn Z. Guyton[b], Peter Kasper[c], Ruthann Rudel[d], Annamaria Colacci[e], Jos Kleinjans[f], Paul Jennings[g]

[a] European Commission, Joint Research Centre (JRC), EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), Ispra, (VA), Italy
[b] Monographs Programme, International Agency for Research on Cancer, Lyon, France
[c] Federal Institute for Drugs and Medical Devices (BfArM), Bonn, Germany
[d] Silent Spring Institute, Newton, United States
[e] Centre for Environmental Toxicology and Risk Assessment, Environmental Protection and Health Prevention Agency, Emilia Romagna Region, Italy
[f] Department of Toxicogenomics, Maastricht University, Maastricht, The Netherlands
[g] Division of Molecular and Computational Toxicology, Amsterdam Institute for Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, HZ Amsterdam, The Netherlands

ARTICLE INFO

Keywords:
Carcinogenicity
Alternative methods
Rodent bioassay
Toxicogenomics
Mechanisms
Cancer hallmarks
CTA

ABSTRACT

There is an increased need to develop novel alternative approaches to the two-year rodent bioassay for the carcinogenicity assessment of substances where the rodent bioassay is still a basic requirement, as well as for those substances where animal use is banned or limited or where information gaps are identified within legislation. The current progress in this area was addressed in a EURL ECVAM- ESTIV workshop held in October 2016, in Juan les Pins. A number of initiatives were presented and discussed, including data-driven, technology-driven and pathway-driven approaches. Despite a seemingly diverse range of strategic developments, commonalities are emerging. For example, providing insight into carcinogenicity mechanisms is becoming an increasingly appreciated aspect of hazard assessment and is suggested to be the best strategy to drive new developments. Thus, now more than ever, there is a need to combine and focus efforts towards the integration of available information between sectors. Such cross-sectorial harmonisation will aid in building confidence in new approach methods leading to increased implementation and thus a decreased necessity for the two-year rodent bioassay.

1. Introduction

The approaches for evaluating the carcinogenic potential of substances, including prioritizing and selecting agents for rodent carcinogenicity studies, differ substantially across sectors. Nonetheless, the two-year rodent bioassay has remained the “gold standard” for carcinogenicity testing for nearly half a century. As from the first OECD Test Guideline release in 1981, the design has remained almost unaltered. Human carcinogens, when tested adequately, have all tested positive for carcinogenicity in one or more animal species (Tomatis et al., 1989; Wilbourn et al., 1986). However, several issues concerning the application of rodent bioassay data to predicting human cancer risks have emerged, with notable challenges in both anticipating the potential human cancer target organs and in quantitative risk estimation, confounded by differences in criteria for interpreting positive findings (Contrera et al., 1997; Knight et al., 2006; Paparella, 2016; Paules et al., 2011; Rudel et al., 2007). For instance, the reproducibility of positive findings in animals (*i.e.*, increased tumours in more than one sex, species or bioassay) is an important consideration in IARC Monograph evaluations (International Agency for Research on Cancer, 2006), which also entail integration with human cancer and mechanistic findings. Moreover, the rodent bioassay, as originally designed, does not take into account windows of susceptibility over the life-time, and so may not have adequate sensitivity to detect agents, such as endocrine active chemicals, that alter susceptibility to tumours (Birnbaum and Fenton, 2003).

The need for clear evaluation guidelines is underscored by the assertion that rodent-specific mechanisms of carcinogenicity, differences in safety margin of exposures, and/or differences in metabolism, confound interpretation of rodent carcinogenicity studies of pharmaceuticals (Friedrich and Olejniczak, 2011; Sistare et al., 2011). Furthermore, these studies are extremely time and resource-consuming and the

* Corresponding author.
E-mail address: raffaella.corvi@ec.europa.eu (R. Corvi).

http://dx.doi.org/10.1016/j.tiv.2017.09.010
Received 2 May 2017; Received in revised form 8 September 2017; Accepted 10 September 2017

high animal burden has raised ethical concerns. Hence, there is a strong demand for rational prioritization schemes as well as for alternative non-animal assessment strategies and methods in the area of carcinogenicity (Annys et al., 2014; Creton et al., 2012; Jacobs and Brown, 2013; Jacobs et al., 2016). Yet, the recourse to currently available alternatives, has been very limited and is quite variable across sectors. Among the main bottlenecks are (i) the difficulties in defining how to meaningfully apply individual *in vitro* tests in the context of other available information, (ii) lack of a complete mechanistic understanding underlying carcinogenicity and (iii) taking the previous two points into consideration what is the regulatory implication of a positive or negative carcinogenicity test result in an *in vitro* assay to the fate of the chemical.

Here we present the outcome of the EURL ECVAM/ESTIV workshop on "the way forward in carcinogenicity assessment" which took place in October 2016 in conjunction with the ESTIV congress in Juan Les Pins.

2. Regulatory background

Regulatory strategies for testing carcinogenicity have diversified significantly across legislations depending on the type of substance, while maintaining two key elements: the testing for genotoxicity *in vitro/in vivo* and the two-year rodent bioassay. For industrial chemicals, requirements are based on a tiered-testing approach and on the annual amount of substance produced, to which potential exposure and degrees of exposure are linked. Carcinogenicity testing is required only for the high tonnage level and mainly for mutagens category 3 (GHS category 3). For all the new plant protection products and non-genotoxic new active biocides, the testing of carcinogenicity is required in two different species. Exposure to these products and their breakdown products is of major concern in occupational settings. On the other hand, for the general population which is exposed to very low doses for long periods of time, the concern is related to persistent metabolites and residues. Carcinogenicity of metabolites and residues is evaluated on a case-by-case basis. Of high concern are also residues of veterinary drugs in food for human consumption. It is a priority of this sector to rely on genotoxicity testing and structural similarities, so that positive results from those studies are further tested. Only when results from genotoxicity tests are clearly negative, no structure alerts are identified and human exposure is negligible, can animal testing be waived. Human medicines are commonly administered at high doses to reach the effective pharmacological dose, with short or chronic exposures. Carcinogenicity testing is performed mainly for drugs for which a chronic administration is foreseen. In this case, a test-battery approach is used starting always with genotoxicity *in vitro* testing followed by *in vivo* testing of genotoxicity and carcinogenicity. In contrast, no *in vivo* testing is allowed since March 2013 for cosmetic ingredients and carcinogenicity is predicted on the basis of alternative approaches only, relying mainly on *in vitro* genotoxicity testing (EC Regulation 1107, 2009; EC Regulation 1223, 2009; EC Regulation 1272, 2008; EC Regulation 1907., 2006; EU Regulation 283, 2013; EU Regulation 528, 2012; ICH S1, 2012; ICH S1A., 1996; SCCS, 2015; VICH GL28, 2005). This limited assessment for carcinogenicity potentially increases the probability of consumers being exposed to cosmetics ingredients which may promote tumours, alter hormone responsiveness of tissue, or influence cancer risk through other non-genotoxic mechanisms.

These cross-sectorial differences in regulation are mostly due to the extent of human health risk for each product use, the level of exposure to humans and the environment, the type of new products developed, economic issues and animal-welfare concerns.

3. Analysis of carcinogenicity testing for regulatory purposes in the EU

The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) has carried out an analysis of carcinogenicity testing requirements and assessment approaches across different sectors within the European Union (Madia et al., 2016). This consisted of a systematic review of the different testing requirements and the number of animals used *per sector*, an estimation of the number of carcinogenicity and genotoxicity studies conducted or waived in respect of the number of substances authorized per sector per year and a review of the type of justifications for waiving the two-year bioassay.

Three Rs initiatives have promoted several changes within regulatory toxicology testing since their first legal embedment in the 1986 EU Directive on the use of laboratory animals. Though, according to the latest figures, there has been a minimal decrease in the animal testing burden used for cancer studies (at least until 2011). In terms of absolute numbers this reduction could be regarded as negligible, as assessment of carcinogenicity *per se* is utilising fewer animals overall in comparison with other regulatory toxicity areas (*e.g.* acute, repro-, chronic toxicity, *etc.*), representing 1% of all toxicity testing (DG ENV Report, 2013). However, in terms of animal welfare, a single cancer study involves a large number of rodents, induces extended suffering, implies a long-lasting period of data analysis and is extremely resource-consuming (Adler et al., 2011). A significant number of carcinogenicity studies are performed in the area of basic research, mainly within academia (DG ENV Report, 2013), though they are usually not referred as to the standard two-year bioassay used for toxicological regulatory purposes.

The two-year bioassay is frequently conducted within the plant protection products sector [> 60%], even if a decrease of the number of substances tested likewise has been observed between years 2011 and 2014 (Madia et al., 2016). The majority of new active substances (10 per year, on average) are tested in a two-year cancer bioassay study or a combined chronic/carcinogenicity rat study, often in combination with a second study in a second rodent species, even though the relevance of the latter has been questioned (Annys et al., 2014; Billinton et al., 2010; Van Oosterhout et al., 1997). The Plant Protection Products Regulation (EC Regulation 1107, 2009) foresees the use of alternative models instead of the second species if scientifically justified, however this is rarely implemented. The carcinogenicity study is waived mainly on the basis of expected limited general population exposure risk, when it is technically not feasible, as in the case of some natural products or microorganisms or on the basis of lack of genotoxicity effect of the substance. In this regard, a conspicuous amount of substances are tested in *in vivo* genotoxicity studies.

The use of alternative approaches has been observed more frequently in the biocide sector which accounts for 12 authorizations per year approximately, 2–5 referring to new substances. The use of read-across data has been reported in several authorization dossiers for either the testing of carcinogenic or genotoxic potential. Opportunities for waiving carcinogenicity testing of biocidal products are similar to those described above for plant protection products. Overall, the two-year bioassay has been performed on 30% of biocidal substances (Madia et al., 2016).

Within the pharmaceuticals sector, a substantial portion of authorized human medicines (on average 35 new substances per year) undergo carcinogenicity testing (as observed either in 2011 or in 2014) and the use of alternative approaches is rarely considered. Within this sector, the two-year bioassay is not conducted for specific classes of therapeutic/diagnostic agents when it is not scientifically relevant or technically feasible. The introduction of specific shorter-term carcinogenicity studies, as the transgenic mouse model, seemed at first to impact positively on the 3Rs, showing promises for more technical specificity and impact on animal number. However, the transgenic model has resulted not to be a real reduction model because of the amount of animals needed for the breeding of the specific knockouts (Daneshian et al., 2015; Ormandy et al., 2011).

In the case of veterinary medicines (on average 10 new substances per year), the percent of authorized substances tested for carcinogenicity were approximately 24% in 2011 and none of the products authorized in 2014 have been tested for this endpoint, because they were