EDITORIAL

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Editorial: Relevance of dog studies for the derivation of health-based guidance values for plant protection products approval

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Under Regulation (EU) 283/2013, setting out the data requirements for pesticides active substance, **short-term oral toxicity** testing in **rodents** (90-day rat study) and **non-rodents** (90-day dog study) species are required to address hazard identification and human safety of plant protection products (PPPs) and to support the active substance approval in the European Union (EU). These animal models allow toxicologists to develop the necessary knowledge to understand chemical hazard and use it in the process of risk assessment with the goal of protecting human population and the environment.

In vivo animal models have historically been the gold standard of safety and risk evaluation and may offer direct evidence of chemical toxicity in a living organism. However, this strength is reduced by the weaknesses of low-throughput, excessive demand of resources, the limited understanding of mechanisms behind the observed toxicity and by ethical reasons. The current trend of extending the toxicity testing, anchored in observational studies, by enhanced mechanistic understanding using human-relevant systems to evaluate biological processes, increases the global effort to allocate more resources to identify the mechanisms of toxicity.

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Moreover, this effort is also in line with the current EU legislation[1] on 3Rs (Replacing, Reduction and Refinement) principles covering the use of animals for scientific purposes.

The necessity to use a rodent species ('first' species) in parallel with a non-rodent species ('second' species) for assessing potential hazardous effects of chemicals used as drugs, pesticides and consumer products, dates back to 1960s (Box and Spielmann, 2005). The main reason why a second mammalian species, phylogenetically different from rodents, was considered, was for the identification of inter-species differences in sensitivity and therefore for the derivation of the health-based guidance values (HBGVs), e.g. acceptable daily intake (ADI), acute reference dose (ARfD) and (acute) acceptable operator exposure ((A)AOEL). The dog was identified as the second testing species mainly because it was already widely used in the USA during the 1950s and was available as a common laboratory breed. Though practicalities such as the larger blood volume of dogs, that allow taking more samples in comparison with mice and rats, and the size of dogs (the Beagle dog in particular), that allows a number of separate physiological and clinical observations, were the main rationale for the selection of this species; other reasons can be recognised considering that dogs are especially suitable for cardiovascular studies due to the resemblance in heart connectivity and size to the human heart (Box and Spielmann, 2005). However, whereas an integrated cardiovascular assessment is mandatory in drug development, in the area of pesticides the only requirement is morphological assessment of the heart.

Therefore, for the approval of **plant protection products**, the scientific rationale of using the dog as 'second' species in the regulatory process has been debated since long time and culminated with the **elimination of the one-year dog** study (OECD TG 452; OECD, 2018) from the data requirements in the EU, the US, Brazil, Canada, Australia and, recently, Japan, leaving the 90-day study (OECD TG 409; OECD, 1998) as the only study available in the dataset for the hazard assessment in a non-rodent species.

The debate is still ongoing and after several decades of using the dog as a 'second' species, the **scientific challenge** remains for unresolved questions:

1) **What is the value of a by default 'second' species when all are a surrogate of humans?**
2) **What is unique of the dog to be of any benefit in the chemical risk assessment and provide a protective ground for human population?**
3) **Are four dogs/sex/group of treatment as per OECD TG 409 really covering the intra- and interspecies variability aspects?**

In this context, the use of dog as 'second' species in regulatory testing of PPPs should be further substantiated. This should be guided not only by practical considerations, but by scientific grounds. To actively contribute to this debate, EFSA reviewed the existing data of the dog studies conducted with the PPP active substances, previously on the European market and/or currently approved by the European legislation and did a **retrospective analysis** of the results on setting of HBGVs for pesticides.

EFSA retrospective analysis of the impact of dog toxicological studies on dietary risk assessment

The current work intended to explore the contribution of dog studies for setting the ADI of active substances used in PPPs.

In this retrospective analysis, 432 past conclusions[2] on PPP active substances, published up to December 2020, were evaluated to determine the basis (type of study, species) for the derivation of the ADI, which defines the regulatory limits on the amount of any chemical that human population can be exposed to over a lifetime without harmful effects.

The ADI has been selected for the current evaluation because, compared to other toxicological reference values such as the AOEL, it is based on oral repeated dose studies and does not require additional extrapolation from external to internal doses. Moreover, consumers are more vulnerable to the effect of active substances considering that they could not wear personal protective equipment (PPE) to mitigate the effect of such chemicals.

[1] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Text with EEA relevance) Text with EEA relevance.
[2] The retrospective analysis considered EFSA conclusions and EU commission assessments on pesticide active substances.

Analysis of available data

The purpose of this retrospective analysis was to explore the relevance of the dog in the process of plant protection products risk assessment, to identify the limitations and the uncertainties in its current role as a test species.

Out of more than 400 active substances registered in Europe, for 101 dog studies were found to be relevant for the setting of ADI, i.e. the point of departure for the setting of the ADI was lower in the dog than in other species. For most of these active substances (96/101 or 96%), **experimental and biological variability, allometric body weight scaling** and **dose spacing** were able to explain the differences observed between rodent and non-rodent species.

A total of five active substances were identified for which dog short-term toxicity study appears to be the most relevant for risk assessment and for which the previous factors were not able to explain the higher sensitivity observed in dogs. However, a clear case where the dog was critical for the assessment of human relevant endpoints of toxicity based on closer physiological similarities with humans was not identified. While assessing the database, it became clear that for some active substances the identification of the dog as the most sensitive species required additional evidence.

Allometric scaling was used as a default screening approach; though, it was recognised that this approach has limitations because it is not considering the impact of the differences in metabolism and ADME characteristics among species. With highly metabolised chemicals it is known that species differences in metabolism are likely to account for differences in target organ toxicity and for the different sensitivity (Martignoni et al. 2006). The recently published Scientific Opinion on testing and interpretation of comparative in vitro metabolism studies (EFSA PPR Panel, 2021), provides a scientific and regulatory framework aimed to illustrate the testing strategy that should be applied to investigate interspecies comparative *in vitro* metabolism and is representing an important step forward for a correct selection of the laboratory species.

Exploring future directions

The current retrospective analyses were limited to the assessment of ADI, however it should be noted that, as also reported in the HSE report (HSE, 2013), dog short-term toxicity studies were mostly utilised in the pesticide area for the **selection of the AOEL** (48% compared to 31% of ADIs).

A more detailed evaluation of the unique characteristic of the dog compared to other species should be conducted for the substances were the dog showed a higher level of sensitivity i.e. lower overall no-observed adverse effect level (NOAEL). Currently, the **comparative *in vitro* metabolism study** is scientifically the most appropriate tool for the selection of the toxicological species and should be included as part of the decision-making process to consider the inclusion of the dog in the regulatory risk assessment of pesticides.

Efforts should focus on identifying which critical scientific and regulatory questions dog studies are addressing, evaluate the cost/benefit of animal use and the impact of dog studies on the paradigm shift from observational endpoints to a more mechanistic reasoning using human-relevant systems to evaluate biological processes. From the regulatory perspective the critical question to be addressed is regarding the impact of the dog on the protection goal as regards human population. Continuation of recommending dog as testing species should be done only if it is beneficial or if alternative approaches are not suitable.

Although the retrospective analysis remains a critical element to understand the usefulness of the dog as part of the data requirement, it is necessary **to elaborate a decision-making process** able to scientifically justify the inclusion or exclusion of the dog in the pesticide risk assessment. The paradigm shift must be convincing for all stakeholders included in the process and a guarantee of safety. A strategy based on a tiered approach and the use of comparative *in vitro* metabolism systems should be therefore explored for concluding on the relevant species selection and eliminate the use of the dog when its inclusion is not representing a real benefit in the risk assessment of pesticides.

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