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SCIENTIFIC OPINION

Scientific Opinion on waiving of the dog studies in the regulatory process of agrochemicals approval

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Abstract

Commission Regulation (EU) No. 283/2013 sets out the data requirements for pesticide active substances and includes short-term oral toxicity testing in rodents (90-day rat study) and non-rodents (90-day dog study) to support human health hazard assessment for pesticide active substances in the European Union (EU). However, the relevance of the 90-day dog study for such assessments has been increasingly questioned. In this context, the EFSA Panel on Plant Protection Products and their Residues (PPR Panel) initiated a self-task mandate to evaluate the contribution of the 90-day dog study to hazard assessment and to explore whether dog studies may be waived from a scientific perspective. This work included a retrospective analysis for the role of dog data in the derivation of health-based guidance values (HBGVs, i.e. acceptable daily intake (ADI) and acceptable operator exposure level (AOEL)) for pesticide active substances in the EU and the development of a tiered assessment strategy to support regulatory decision-making for new active substances. The evidence obtained in the retrospective analysis showed that, in 97% of the cases for ADI and 95% for AOEL, observed interspecies differences in established no observed adverse effect levels (NOAELs) were attributable to experimental and biological variability, differences in study design and/or allometric scaling, rather than reflecting a genuine dog-specific sensitivity. Further analyses of modes of action (MoA); absorption, distribution, metabolism and excretion (ADME); and toxicokinetic (TK) profiles across species were conducted. Based on the retrospective analysis, a tiered assessment strategy was defined to decide whether for a new active substance, the 90-day dog study can be waived. A minimum data set and scientific criteria were defined for this assessment strategy, which includes integration of physiologically based kinetic (PBK) modelling and human-relevant data. The PPR Panel concludes that this strategy provides a robust basis to support the waiving of dog studies when scientifically justified and supports the implementation of new approach methodologies (NAMs) in the regulatory risk assessment of pesticide active substances.

KEYWORDS

new approach methodology (NAM), pesticide active substances, physiologically based kinetic (PBK) modelling, waiving dog studies

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SUMMARY

Commission Regulation (EU) No. 283/2013 sets out the data requirements for pesticide active substances. Among others, short-term oral toxicity testing in rodents (90-day rat study) and in non-rodents (90-day dog study) are required to address human health hazard assessment of pesticide active substances to support their approval in the European Union (EU).

The added value of the 90-day dog study for these hazard assessments has been questioned. A retrospective analysis of the active substance approval process previously conducted by EFSA underlined that, in 95% of the evaluated cases (96 out of 101 active substances), the dog was not the most sensitive species used for setting the health-based guidance values (HBGVs).

In this context, the EFSA Panel on Plant Protection Products and their Residues (PPR Panel) was self-tasked to develop a Scientific Opinion with two objectives ('Terms of Reference' or ToR): [1] to review and update the aforementioned retrospective analysis (ToR1) with a broader database of active substances and [2] to develop a tiered assessment strategy that can support the decision-making of whether waiving of the dog studies in the regulatory process can be scientifically justified (ToR2). To this end, a dedicated Working Group of the PPR Panel (hereafter referred to as 'PPR Panel WG') was established.

For ToR1, the retrospective analysis was expanded to 461 active substances registered in Europe. Among these 461 active substances, dog studies were considered relevant for the setting of HBGVs in 288 cases: 107 (23%) for acceptable daily intake (ADI) and 183 (40%) for acceptable operator exposure level (AOEL), where dogs showed greater sensitivity to toxicity than other species tested.

For these cases, an in-depth analysis was performed to assess whether differences are due to genuine dog-specific sensitivity or whether they could be attributed to other factors, including experimental and biological variability, differences in study design (e.g. dose spacing or selection) or whether they could be explained by allometric scaling.

Higher sensitivity of dog due to experimental variability was considered for cases when the $NOAEL_{other\ species}/NOAEL_{dog}$ is ≤ 3 (ADI: 83 cases; AOEL 183 cases). Higher sensitivity of dog due to dose spacing was considered for cases when the ratio of $NOAEL_{other\ species}/NOAEL_{dog} > 3$ but the $NOAEL_{other\ species} < LOAEL_{dog}$ (a further 7 cases for ADI and 17 cases for AOEL). For cases where the lower NOAEL in dog is based on a 1-year or 2-year toxicity study, the NOAEL from 90-day dog study (when available) was retrieved. If the NOAEL from the 90-day dog study was in the same range as the lower NOAEL from other species (i.e. ratio of $NOAEL_{other\ species}/NOAEL_{90\text{-}d\ dog} \leq 3$), no further analyses were considered needed as only the 90-day study in dog is required according to Regulation (EU) No. 283/2013 (a further 7 cases for ADI and 6 cases for AOEL). Human equivalent doses (HEDs) were calculated to evaluate whether differences between dogs and other species could be explained by allometric scaling (a further 7 cases for ADI and 7 cases for AOEL). Overall, in 97% of the cases for ADI and 95% for AOEL, the observed interspecies differences in the established no observed adverse effect level (NOAEL) between dog and other species such as rat were not reflecting genuine dog-specific sensitivity.

This outcome served as a basis for further investigations into the mode of action (MoA); absorption, distribution, metabolism and excretion (ADME) characteristics; and toxicokinetic (TK) profiles of the relevant active substances across species. The findings from the updated retrospective analysis supported the setting of a minimum data set and scientific criteria required to structure a tiered, weight-of-evidence (WoE)-based approach to determine whether dog toxicity data are needed from a scientific perspective. Based on this retrospective analysis, a tiered assessment strategy (ToR2) was developed to support the decision-making process for pesticide active substances, with the aim of ensuring that HBGVs remain protective while avoiding unnecessary dog toxicity testing. The assessment strategy consists of two preliminary phases and a flowchart. Preliminary phase 1 considers in vitro comparative metabolism studies and the toxicological mode of action of the active substance and allows an early decision on the possible waiving of dog toxicity studies. A dog study may be waived in case the dog shows a dissimilar in vitro metabolite profile compared to humans and/or if the active substance belongs to a well-defined chemical class for which sufficient toxicological MoA data are available. For substances with an MoA for which the dog is known to be less sensitive than rats (rodents), a dog study may be waived. For substances with an MoA for which the dog is more sensitive than rodents, dog toxicity studies may also be waived but with further consideration of an extra uncertainty factor, depending on the available data and context of the assessment. Preliminary phase 2 evaluates whether the rat is considered an adequate test species for the human health hazard assessment regarding internal exposure upon oral intake based on the information available from in vivo ADME studies in rats and in vitro human Caco-2 permeability data. If waiving of the dog study cannot be concluded in preliminary phase 1 and the rat is identified as an adequate test species in preliminary phase 2, the flowchart needs to be followed, focusing on interspecies comparison of internal exposure supported by physiologically based kinetic (PBK) modelling. If it can be concluded from the analysis that the dog does not provide added value for hazard assessment based on internal exposure considerations, the requirement for a dog toxicity study may be waived.

To assess the feasibility of employing the assessment strategy, data for four active substances were available for case studies. In three case studies, it could be demonstrated with medium to high confidence that hazard assessment using primarily rat toxicity data without dog toxicity testing could be performed. Limitations and uncertainties were identified while testing the assessment strategy and are discussed in the Scientific Opinion.

Key recommendations are made in this Scientific Opinion to facilitate the fulfilment of the necessary conditions to effectively run the assessment strategy for waiving purposes. The proposed tiered assessment strategy fosters the use of New Approach Methodologies (NAMs) in the regulatory risk assessment of pesticide active substances, which is fully in line with EFSA's Strategy 2027[1] and the European Commission's roadmap towards phasing out animal testing.[2]

[1] https://www.efsa.europa.eu/sites/default/files/2021-07/efsa-strategy-2027.pdf.

[2] https://single-market-economy.ec.europa.eu/sectors/chemicals/reach/roadmap-towards-phasing-out-animal-testing_en.

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1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background

Under Regulation (EU) 283/2013 (European Commission, 2013), setting out the data requirements for pesticides active substances, 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).

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 the 1960s (Box & Spielmann, 2005). The main reason why a second mammalian species phylogenetically different from rodents is used, was to identify interspecies differences in sensitivity and therefore for the derivation of the health-based guidance values (HBGVs), e.g. acceptable daily intake (ADI), etc.

For the approval of PPPs, the scientific rationale of using the dog as 'second' species in the regulatory process has been debated for long time and culminated in the elimination of the one-year dog study (OECD TG 452; OECD, 2018) from the data requirements in the EU, leaving the 90-day study (OECD TG 409; OECD, 1998) as the only study available in the data set for the short-term repeated dose toxicity study in a non-rodent species.

PREV Unit and Plant Protection Products and their Residues (PPR) Panel members have reviewed the existing data on dog studies conducted with the pesticide active substances previously marketed in the EU and/or currently approved by the European legislation. A retrospective analysis of the results on setting of HBGVs (i.e. ADI) for pesticides was performed. An overview of the work is published as editorial in the EFSA Journal (Panzarea et al., 2022).

The current project aims to continue contributing to the debate by reviewing/updating existing data to develop a tiered testing strategy, recommending the possible waiving of dog studies in the regulatory process when deemed scientifically justifiable. In fact, from the retrospective analysis (Panzarea et al., 2022), for the 95% of cases in a database of 101 substances, the dog was not the most sensitive species used for setting the HBGVs. However, this project aims to go into the details of the previous analysis and explain the relevance of the dog for the cases where this species was the most sensitive one.

The outcome of the project will contribute to identifying which critical scientific and regulatory questions dog studies are expected to answer, if any. In a broad perspective, the project will provide evidence to assess the economic cost/benefits of the dog studies contextualizing the ethical use of animals in experimental toxicology. It will also assess the impact of dog studies on the paradigm shift from observational endpoints to a more mechanistic reasoning, using human-relevant test systems to evaluate biological processes.

This project aligns with broader objectives, including complementing the European Commission's roadmap[3] to accelerate phasing out of animal studies in chemical risk assessment, integrating the use of New Approach Methodologies (NAMs) in the regulatory process as per the EFSA Strategy 2027 and, fostering collaborations with EFSA's stakeholders involved in analogous projects.

1.1.2 | Terms of Reference

The PPR Panel is requested to:

1. Review/Update the existing data (starting from the retrospective analyses conducted in 2022).[4]
2. Develop a tiered testing strategy to support the recommendation of waiving of the dog studies in the regulatory process, when deemed scientifically justifiable.

1.1.3 | Interpretation of the Terms of Reference

In its interpretation of the Terms of Reference (ToR), the PPR Panel WG determined that the main objective of this mandate is to advise risk managers and EFSA stakeholders on the conditions under which toxicity studies in dogs might be considered dispensable in the approval process of pesticide active substances and under what circumstances their inclusion might be justified. Therefore, the intended goal of this work is to provide an assessment strategy to determine whether dog toxicity studies are necessary for the human health hazard assessment of pesticide active substances or whether they can be waived with scientifically robust justification.

[3] https://ec.europa.eu/commission/presscorner/detail/en/ip_23_3993.

[4] https://doi.org/10.2903/j.efsa.2022.e200923.

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In addressing ToR1, the PPR Panel WG reviewed and updated existing evaluations, starting with the previously conducted retrospective analysis on this topic (Panzarea et al., 2022) to ensure that current regulatory assessments are based on the latest scientific evidence. The emphasis of the updated analysis was placed on assessments of the effects of repeated exposure because it is widely understood that identifying toxicity from repeated exposure is the main objective of using dog toxicity studies. Occasionally, effects in repeated dose oral toxicity studies are used as a surrogate for acute effects but do not necessarily reflect acute toxicity. In particular, the acute effects most commonly observed in dogs (e.g. diarrhoea, vomiting, local gastrointestinal irritation) are considered to be a result of dog-specific induced emesis/local gastrointestinal toxicity rather than being effects of the systemic toxicity of the test substance, meaning these acute effects in dogs are not suitable for extrapolation to humans. Furthermore, these effects are dose-limiting as less of the substance potentially reaches systemic circulation. The outcome of ToR1 is addressed in Section 4.1.

Regarding ToR2, the PPR Panel WG interpreted the development of a tiered testing strategy, as mentioned above in Section 1.1.2, keeping in mind the broader context of reducing or phasing out animal studies. Specifically, for this mandate, the PPR Panel WG developed a tiered assessment strategy to support the potential waiving of dog studies in the regulatory approval process of pesticide active substances while ensuring robust human health safety assessments. This approach critically evaluates existing and relevant data to determine if dog toxicity studies are truly necessary to adequately identify and characterise human health hazards. To achieve this, the strategy defines the minimum set of data required for assessment, prioritising the incorporation of NAMs. The outcome of ToR2 is addressed in Section 4.2.

To test the tiered assessment strategy, proof-of-concept case studies were performed, using empirical data to substantiate and/or refine the decision framework. The Scientific Opinion defined the conditions under which dog toxicity studies might not be required based on a critical analysis of the available scientific data.

In the context of this Scientific Opinion, it should be emphasised that the term 'agrochemicals' strictly refers to the active substances undergoing or having undergone approval review process in the EU. The developed Protocol (Annex A) and the resulting Scientific Opinion for this mandate are independent of the pesticide peer review process and are not intended to modify regulatory decisions.

The economic cost/benefit of waiving dog studies, as mentioned in the Background (Section 1.1.1) of this Scientific Opinion, is not elaborated in this document, as the PPR Panel WG deemed it of foremost importance to focus on scientific and regulatory argumentations for waiving dog studies.

Abbreviations used in this Scientific Opinion can be found in a separate chapter at the end of the document. Additional background information on specific scientific topics (e.g. toxicokinetics and toxicodynamics) relevant for this mandate can be found in Appendices A and B, and protocols as well as outputs generated/provided by the PPR Panel WG can be found as Annexes as supplementary documents.

The draft Scientific Opinion developed by the PPR Panel WG underwent public consultation.[5] Also, a stakeholders' event was organised (Annex F). Feedback received from stakeholders during the event and through the public consultation was considered for the finalisation of this Scientific Opinion (Annex G).

2 | DOG AS AN EXPERIMENTAL ANIMAL MODEL

This section provides background and context considered pertinent to the ToRs addressed in the current Scientific Opinion. In particular, it outlines the historical reasons for using Beagle dogs as animal models in toxicity testing and highlights some critical elements that have led to the re-evaluation of their continued use in toxicology, focusing on relevant aspects of canine physiology and pathology.

First and foremost, it is important to acknowledge that no commonly used laboratory species (e.g. dogs and rodents) can fully represent the complexity and variability of the human population and that every tested mammalian species has apparent physiological and/or anatomical differences from humans.

Notwithstanding, under the pesticide regulatory framework, the rat is the elective primary animal model to cover and address the key hazard endpoints, e.g. carcinogenicity, reproductive and developmental toxicity, neurotoxicity, of an active substance and its use is regulatorily justified for both scientific and practical reasons. The interspecies differences between humans and any mammalian species used for toxicity testing (in this case, rat) are critically considered by risk assessors when assessing the potential human health hazard of a substance.

The use of a second mammalian species for hazard assessment of chemicals has traditionally aimed to evaluate and address uncertainties regarding interspecies variability. However, selecting two species for toxicity testing does not necessarily reduce uncertainty regarding potential human health risks and there is emerging evidence to suggest that a second species may not be required in many cases for the hazard assessment (Bishop, Brescia, et al., 2023; Bishop, Dellarco, & Wolf, 2023; Janer et al., 2008; Linke et al., 2017). Nevertheless, there have been some circumstances, such as those in drug development, that justify the use of a second species like dog for safety assessment, but the decision is based on scientific a priori knowledge of the species differences in kinetics or toxicity (Bower et al., 2022).

The OECD Test Guideline (TG) 409 (Repeated Dose 90-day Oral Toxicity Study in Non-Rodents), which is the test protocol relevant for the implementation of Commission Regulation (EU) No 283/2013, indicates that the 90-day study in non-rodent species provides information on the possible health hazards likely to arise from repeated exposure over a period

[5] https://connect.efsa.europa.eu/RM/s/consultations/publicconsultation2/a0lTk000005lQFV/pc1650.

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of rapid growth and into young adulthood (although most of the time dogs would still be in the peripubertal/pubertal sexual maturity stage at the end of the study). The study can also provide information on potential toxic (adverse) effects, target organs and the possibility of accumulation and can identify a no observed adverse effect level (NOAEL) of exposure for establishing HBGVs. It is of note that, in paragraph 4 of the OECD TG 409, it specifies the following conditions, in which the second species should be used:

- 'where effects observed in other studies indicate a need for clarification/characterisation in a second, non-rodent species, or
- where toxicokinetic studies indicate that the use of a specific non-rodent species is the most relevant choice of laboratory animal, or
- where other specific reasons justify the use of a non-rodent species'.

Therefore, in contrast to the Commission Regulation (EU) 283/2013 (1 March 2013 and current consolidated text 21/11/2022), where testing in a second species for short-term toxicity is required by default, the OECD TG programme recommends the use of a second non-rodent 90-day study only under specific circumstances and when the conduction of the study is triggered after toxicokinetic (TK) and toxicodynamic (TD) considerations.

2.1 | History and traits of the Beagle dog

The dog, *Canis familiaris*, is likely to be one of the oldest domesticated animals with skeletal remains discovered dating back to the middle Stone Age (6000–8000 B.C.) (Scott, 1964). Man discovered that some dogs had specific abilities that could better serve his needs, and by selective breeding, the desired traits were fixed and consistently transmitted to the progeny. Today, approximately 400 different canine breeds are recognised. They have remarkable genetic variations, underpinning the broad spectrum of physical, physiological and behavioural traits that are typical of the contemporary canine breeds.

Even though the dog has a long history of use in research, researchers only became cognizant during the latter half of the 20th century that the lack of standardisation of using dogs as experimental model, particularly regarding the health status, was an issue. The use of non-standardised animals was considered a relevant hurdle to define what can be considered the 'normal' state of health. The consequence was that researchers were obliged to select a breed, originally selected for other purposes, but acceptable to experimental procedures. Among the various breeds considered for research, one survey indicated the Beagle as the breed of choice by a factor of 20:1[6] on selected characteristics (e.g. weight, size, temperament, etc.) (Scott, 1960).

The name Beagle is thought to derive from the French word *beagle* meaning bugler. However, the English were the main breeders of the Beagle as a hunting dog with an excellent scenting ability, and the breed standard for the Beagle was first described in the early 19th century (Taplin, 1803). Modern-day Beagles are thought to have descended from Talbot Hounds, large white scent-hounds that accompanied William the Conqueror into Britain during the 11th century. Another now-extinct breed that may have contributed some DNA to the modern Beagle is the Irish Kerry Beagle, small black-and-tan dogs similar to a Bloodhound. The name Beagle first appeared in English literature in 1475. It was mentioned in the works of no lesser literary lights than Chaucer and Shakespeare. 'She's a Beagle, true-bred, and one that adores me', says Sir Toby Belch in Twelfth Night. Queen Elizabeth I was among the many Beagle enthusiasts who owned one of the variations on the basic design. Her 'pocket Beagles' stood about 9 inches tall. These tiny hounds were also known as 'singing Beagles' or 'glove Beagles'. Beagle-like dogs appeared in America sometime before the Civil War. However, the breed was not firmly established until the 1880s. In 1885, a Beagle named Blunder became the first to be registered by the American Kennel Club (AKC). In the 1890s, the recently formed National Beagle Club held its first field trials. The Beagle therefore became popular in the United States at the end of 19th century and was one of the most popular dog breeds in the United States around the 1950s.

The most desirable qualities of the Beagle as an experimental breed are its medium size, moderate length coat in two or more colours, even temperament, adaptability to living in groups and representative conformation, as well as the lack of need for cosmetic surgery.[7] The Beagle's excellent disposition and friendly personality are great assets of the breed because special handling is often necessary with minimal restraint in several experimental procedures. They rarely show aggressiveness, timidity or shyness. In the past, variations in size and body weight, shedding and loud bark were initially recognised as faults; while shedding and barking remain unresolved issues, the size of the Beagle male and female dogs has been much standardised nowadays, and these issues are no longer perceived as faults.

2.2 | The use of Beagle dog in regulatory toxicology studies

For the toxicity or safety testing of pesticides or drugs, respectively, evaluation of defined toxicological endpoints, such as short-term oral toxicity in 90-day studies, has traditionally required testing in two mammalian species, typically one rodent and one non-rodent species.

A comparison of the experimental design in the oral 90-day (guideline) study between dog and rat is shown in Table 1.

[6]Factor 20:1 refers to the number of characteristics (positive: negative) that led to the selection of Beagle dogs as relevant species used in toxicological testing.

[7]In veterinary use, the term 'cosmetic surgery' infers the cleaning and shaving of the operational area.

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TABLE 1 Comparison of the experimental design in the repeated dose 90-day oral toxicity study between rat and dog.

	OECD TG 408 (90-day rat) adopted: 25 June 2025 (OECD, 2025)	OECD TG 409 (90-day dog) adopted: 21 September 1998 (OECD, 1998)	Considerations
Number of animals	10/sex/dose + recovery (optional 5/sex for control and high-dose groups)	4/sex/dose + recovery (optional 4/sex for control and high-dose groups)	The number of animals together with the lower level of standardisation of the dog population compared to rat represents an overall uncertainty in the statistical analysis.
Number of doses	3 dose levels and a concurrent control	3 dose levels and a concurrent control	–
Route of administration	By oral gavage, incorporated in the diet or dissolved in drinking water (mostly by diet for pesticides)	By diet or in the drinking water or by capsule or gavage	The test item is often given to dogs by capsule or gavage, which is not a typical human exposure route.
Age at start	Young adult animals (sexually mature, e.g. before the start of the experiment, animals are 9 weeks old)	Young animals (can be immature, early or late peripubertal age, e.g. at 4–6 months and no later than 9 months of age)	The sexual maturation in dog is variable, and the assessment of the male reproductive system is a relevant limitation in this species (see Section 2.2.2).
Body weight, food and water consumption	Yes, on all animals weekly	Yes, on all animals weekly	–
General clinical observation	Twice a day (usually at the beginning and end of each day)	Once a day (twice a day to check for any morbidity or mortality)	–
Detailed clinical observation	Weekly (in open arena)	Weekly (in open arena)	–
Functional observation battery	Performed on high dose and control animals by default	Not performed	Although technically feasible, a functional observation battery to monitor sensory and motor functions is generally not performed in dog with the additional limitation of the low number of animals by dose group.
Haematology	Yes, on all animals	Yes, on all animals	–
Clinical biochemistry	Yes, on all animals	Yes, on all animals	–
T3, T4 and TSH	Yes, on all animals	No (optional)	–
Total cholesterol, LDL, HDL	Yes, on all animals	No (optional)	–
Urine analysis	Optional	Yes, on all animals	–
Macroscopic examination at necropsy	Yes, on all animals	Yes, on all animals	–
Organ weight at necropsy	Yes	Yes	On a standard list of organs for both species.
Histopathology	Yes, on a standard list of organ/tissues in control and high dose animals	Yes, on a standard list of organ/tissues in all animals	In dog, the size of the organs can limit the representativeness of the analysis.
Sperm parameters analysis	Optional (may be restricted to control and high-dose males)	No	–
Staging of the testicular seminiferous epithelium	Yes, on all control and high dose animals	No	–
Oestrous cycle at necropsy by vaginal smear cytology	Yes, on all animals	No	–

The dog (namely the Beagle) is considered the elective second mammalian species and has been extensively used for hazard assessment for regulatory applications. Considering all in vivo animal models, using the dog for toxicity testing has both advantages and limitations, the latter including small sample size resulting in low statistical power and a lack of suitability for the assessment of biological products (e.g. antibodies, for which the elective species would be the non-human primate). This section is not intended to list the advantages and the limitations of using the dog in experimental studies, as

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several seminal publications and books have already discussed this (Morton, 1998; Namdari et al., 2021), but rather to focus on some critical elements that should be considered when thinking about any potential added value of including the dog in toxicity studies for the purpose of characterising the toxicological profile of a substance. Two critical elements worth discussing here are (1) the dog's gastrointestinal (GI) physiology and its impact on the internal exposure and (2) certain specific histopathological characteristics of the Beagle dog that can affect hazard characterisation.

Discussions on species-specific enzymatic activities (e.g. cytochrome P450 enzymes (CYPs)) and renal clearance (e.g. glomerular filtration rate, transporters activities) are not described in this chapter because these are not considered to be unique characteristics of the dog, but rather general factors impacting species and individual toxic sensitivity via toxicokinetic differences.

2.2.1 | A comparison of dog and human gastrointestinal (GI) physiology

The acute effects most commonly observed in dogs (e.g. diarrhoea, vomiting, local gastrointestinal irritation) are considered to be a result of dog-specific induced emesis/local gastrointestinal toxicity rather than being effects of the systemic toxicity of the test substance (Clark & Steger-Hartmann, 2018; Elwood et al., 2010) and are considered of limited relevance to humans.

The digestive tract of the dog is adapted for a diet typical of carnivores, focused on the rapid breaking down of proteins. Dogs have a shorter small intestine and a longer large intestine relative to their size; this is different from humans, where the longer small intestine and the presence of specialised enzymes (e.g. amylase) allow for an efficient absorption of nutrients represented by a mix of plant and animal components. Although anatomical differences among the two species exist, an advantage of including dog studies in hazard assessment is the relatively comparable ADME characteristics between humans and dogs, making the evaluation easier compared to other animal species. However, despite the large number of studies performed, significant discrepancies still exist between the oral bioavailability observed in dogs and that in humans. The two main aspects of the GI physiology expected to affect the absorption and bioavailability of chemical substances are the transit time/motility of the substance and the pH of the GI tract, which are further elaborated in the section below. Species-specific differences in GI physiology are expected to influence the time profile and/or the extent of absorption of a chemical substance, e.g. showing differences between dogs and humans (Dressman, 1986; Smeets-Peeters et al., 1998). It should additionally be noted that variations among breeds that can influence absorption of chemical substances also exist in canine gastrointestinal physiology (Bourreau et al., 2004; Oswald et al., 2015).

2.2.1.1 | *Residence time in the stomach*

Most of the absorption of a substance in dogs occurs in the small intestine, with some exceptions related to absorption in the colon (e.g. short- and medium-chain fatty acids and octanoic acid). However, before entering the small intestine, feed and chemical substances must transit through the stomach, where several physiological processes occur, noting that the dog does not have amylase in the saliva. The residence time of a chemical substance in the stomach depends on several factors. These include the administered form (liquid vs. solid), the volume of liquid administration, the particle size and the fasted vs. the postprandial (recently fed) states. In the fasted state, the motility of the upper GI tract consists of three main phases (phase I with no contractions, phase II characterised by intermittent contractions and phase III characterised by regular rhythmic contractions) and feeding results in changes in the motility pattern. In the stomach, motility is characterised by regular tonic contractions, pushing food towards the antrum while mixing it with the gastric secretion. The fasting motility pattern is resumed when the meal has been emptied from the stomach.

These patterns are qualitatively similar between dogs and humans, but quantitative differences exist, affecting absorption. Fine particles are emptied at a similar rate to digestible solid in the fed state and the dependency of emptying on the particle size in the two species is relevant. The motility patterns of the GI tract are thought to be similar between the two species and monolithic dosage forms are expected to be similar in terms of residency time. Indeed, fine particles will empty from the stomach with fluids, whereas particles too large to empty with fluid will exit in conjunction with Phase III activity. There is a relationship between particle size/density and rate of emptying relative to the meal. Overall, this general trend is similar between the two species, meaning particles of less than 0.5 mm should empty with fluid, particles between 0.5 and 3 mm should empty during the course of the meal and clearance of particles larger than 4.5 mm is expected to be delayed until the meal has emptied (Gruber et al., 1987; Meyer et al., 1985).

Assessing the transit time along the intestine is more complex than in the stomach and data are less consistent (Koziolek et al., 2019). In humans, transit generally takes 3–5 h, independent of the particle size or whether in a fasted state, but transit can take longer after ingesting a heavy meal. The effect of a heavy meal is not known in dogs, but it is known that particle size can affect the transit rate. This relationship has not been confirmed in human. A mean small intestine residence time of 238 ± 14 min was reported for human, consistent with the transit time under fasting or light meal conditions (Dressman, 1986). Transit time in dog is less than half that in humans and more variable, ranging from 15 to 206 min, possibly indicating that absorption of chemical substances in dogs is more variable and less complete, but exceptions can exist (Koziolek et al., 2019).

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2.2.1.2 | *Role of pH in the GI tract in absorption*

The pH of the GI tract is the second relevant factor affecting absorption of chemical substances. For passively absorbed substances, the non-ionised form is generally better absorbed than the ionised form. The pH of the GI tract can, therefore, dictate the rate of absorption of chemical substances with dissociation constraints in the physiological pH range. For lipophilic chemical substances, the pH of half-maximal absorption should be considered instead of the pKa. For non-lipophilic chemical substances, the pH of half maximal absorption and the pKa are expected to be similar. If the pH of half-maximal absorption is in the range of the pH observed in the GI tract, changes in pH due to feeding, a disease state or species differences will affect bioavailability. Variations in the GI pH can also affect the ionised status of actively absorbed chemical substances, and they can influence the dissolution state, particularly affecting poorly soluble chemical substances.

Gastric pH in the fasted dog is about 1.5 ± 0.04 (ranging 0.9–2.5), whereas, in humans, it ranges from below 1 to 3.2, with a mean maximum pH of 2.1 and a mean minimum pH of 1.3. After meal ingestion, there is a rise in pH in humans due to the buffering effect of food, followed by a rise in gastric secretion with a gradual return to pre-meal values in 60–90 min. The acid peak in humans occurs around an hour after meal and can possibly also be affected by the order of fluid vs. solid administration. The buffering effect is not observed in dogs and there is no change in pH after the first postprandial hour, which can be explained by the higher acid peak observed in dogs, minimising the buffering effect of feed. Therefore, while the gastric pH is similar between humans and dogs in the fasted state, the initial postprandial pH peak is absent in dogs, making the pH response to meals less predictable and more variable.

The pH in the intestinal lumen of dogs is consistently 1 unit higher than in humans when normalised to gastric emptying. This is mainly due to the lower basal gastric secretion while the concentration of pancreatic bicarbonate (representing the main buffering system in the small intestine) is similar between the two species. The pancreatic bicarbonate output is, however, more variable in dogs, and in this species, the rate of bicarbonate secretion in the fasted state is higher than the basal acid secretion. For humans, the opposite is true. Therefore, it is likely that chemical substances with a pH at half-maximal absorption in the pH range between 5 and 7 may be absorbed at different rates when comparing the two species. The intestinal pH of dogs has a wider pH range than that of humans, and this would affect the variability in absorption of poorly absorbed chemical substances with a half maximal absorption pH in this range (Table 2). These aspects are expected to affect the absorption and bioavailability of chemical substances, limiting the suitability of the dog as a protective model species (Martinez & Papich, 2009).

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TABLE 2 Overview of the similarities and differences between human and dog and impact on absorption.

	Human	Dog	Impact on absorption
Residence time in the stomach	Similar motility pattern between the two species Food particles < 0.5 mm get quickly emptied with fluids Particles between 0.5 and 3 mm get emptied during the course of the meal Particles > 4.5 mm get emptied after the meal has emptied from the stomach		Some quantitative differences on motility between the two species exist, which can affect absorption rate of the two species
Transit time along the intestine	3–5 h (fasted or after ingestion of a light meal) Longer than 5 h after ingestion of a heavy meal	15–206 min Transit time after ingestion of a heavy meal unknown	Transit time in the intestine in dogs is highly variable, leading to more variable and less complete absorption of chemicals
Gastric pH	Range 1.0–3.2 pH rises after meal ingestion ('buffering effect')	Range 0.9–2.5 Buffering effect of food not observed	Dogs do not show buffering effect of food as humans do, making the pH response to food in dogs more variable
Intestinal pH (lumen)	Higher gastric acid basal secretion than pancreatic bicarbonate secretion	Wider range than humans but 1 unit higher than that in human Higher rate of pancreatic bicarbonate secretion (but highly variable) than gastric acid basal secretion	Absorption of chemical substances with pH at half maximal absorption ranging from 5 to 7 might be highly variable in dogs and different between the two species due to different patterns of output in the GI tract

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2.2.2 | Histopathological traits

The spontaneous (i.e. not experimentally induced) background incidence of histopathological changes in dogs has been widely described in several dedicated papers and, more recently, collected in an INHAND publication (Woicke et al., 2021) with the purpose of providing a standardised nomenclature for classifying lesions observed in most tissues and organs from the dog that are examined in toxicological studies. A more comprehensive understanding of the spontaneous histopathological changes in Beagle dog can be retrieved from the abovementioned INHAND publication.

Two conditions commonly observed in the Beagle dog as an experimental species, namely idiopathic canine polyarteritis and particular features of the male reproductive system, must be interpreted with caution when evaluating dog toxicity studies for the hazard characterisation of pesticide active substances as they can confound histopathological diagnosis of effects of the substance being tested. This consideration comes primarily from the regulatory experience. For example, the presence of isolated cases of idiopathic canine polyarteritis could represent a relevant confounding factor when a distinction between background pathology and treatment-related findings is necessary, or, as in the case of the histological characteristics of the dog reproductive system (when matched with the peripubertal status), these cases could seriously limit the use of the dog in the assessment of male reproductive toxicants.

Idiopathic canine polyarteritis is also known as Beagle pain syndrome or canine juvenile polyarteritis syndrome. Understanding and morphologically differentiating the spontaneous occurrence of this disease from chemically induced vascular changes is relevant in toxicological studies because chemically induced vascular pathology can have some common traits with the Beagle idiopathic canine polyarteritis. The aetiology and pathogenesis of this disease remain unknown and the site predilection (i.e. affected vessels), dose–response pattern and clinical and changes in laboratory clinical chemistry and haematological parameters (e.g. neutrophilic leucocytosis, hyperfibrinogenaemia and hypoalbuminemia) should be considered to differentiate the spontaneous vascular syndrome from a treatment-related effect. Idiopathic canine polyarteritis usually shows prominent transmural to periarterial inflammation and haemorrhages are seldomly observed; it will typically affect arteries of the heart and of many other tissues and it is not associated with haemodynamic changes, whereas a constellation of clinical signs like pyrexia, anorexia, reluctance to move, cervical rigidity and pain are variably reported. Elevated polymorphonuclear cell count and concentration of immunoglobulin A (IgA) and acute phase proteins are frequent concomitant changes at haematological and clinical chemistry analysis. However, the background condition can be exacerbated following treatment with vasoactive or immune modulators substances (Clemo et al., 2003; Hogenesch et al., 1995; Son, 2004).

The other relevant condition refers to the male reproductive system of dogs used in toxicological studies. Dogs used in toxicity studies can be 5–6 months of age at the start of the test, but sexual maturity occurs from 7 to 12 months of age, depending on the source of the animals. The use of animals with sexually immature or peripubertal reproductive organs is not a sensitive means to detect potential treatment-related effects on adult reproductive tissues. Dog spermatogenesis is less organised and less synchronised than in rodents, and special staining (i.e. PAS staining) of the acrosome is less specific in classifying canine spermatogenesis. In addition, the canine male reproductive tract is not synchronised when considering the rate of maturation of the testes, epididymides and prostate. Indeed, maturation of the prostate may be delayed in relation to the state of sexual maturation in the testes or vice versa. In addition, detailed stage-dependent evaluation is also challenging in the dog because the release of mature spermatids may occur over two stages and dogs have a higher incidence of spontaneous findings (e.g. hypospermatogenesis, swollen spermatocytes, multinucleated giant cells or changes associated with peripubertal development) compared to the rat. Germ cells in dogs seem to lack androgen and follicle stimulating hormone (FSH) receptors, and therefore, the target for the hormonal control is likely via the stromal cells. Consequently, capturing testicular toxicants in dogs can be challenging or even impossible, and the use of male dog for the assessment of the endocrine-disrupting properties of active substances has often posed major limitations in the assessment and using the dog for this purpose should be taken with caution (Greaves, 2011; Soares et al., 2009).

3 | DATA AND METHODOLOGIES

3.1 | Problem formulation

In line with the ToRs and the draft framework for protocol development for EFSA's scientific assessments (EFSA Scientific Committee, 2023), the following assessment question was raised in the protocol (Annex A) and addressed:

1. What are the criteria to determine whether dog toxicity studies should be included as part of the data requirement for the approval of active substances included in plant protection products?

The sub-questions were subsequently formulated based on the assessment question above and the ToRs as described below:

For the ToR1, the retrospective analysis performed by EFSA in 2022 was expanded to include more active substances. For the update, additional data/information on the active substances were collected (**Section 3.2**) and extracted (**Section 3.3**), and the PPR Panel WG then performed a critical review of certain active substances (see **Sections 3.4** and **3.5** for more details) with the aim of addressing the following sub-questions:

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1.1 Are the differences in NOAELs between species due to allometric body weight scaling, taking into consideration the ADME (absorption, distribution, metabolism, excretion) properties of the substances (if possible) and excluding experimental design differences? Are there alternative approaches to allometric body weight scaling that can be used to explain the divergence in reference points across species?
1.2 Are there specific factors that explain the observed differences between animal species used in experimental toxicity testing of active substances where the dog is identified as the critical species for the selection of reference points?

Based on the outcomes of ToR 1, the PPR Panel WG clarified the specific circumstances (i.e. scientific criteria; **Section 3.6**), under which waiving dog studies in the regulatory assessment of active substances could be scientifically justifiable. For ToR 2, the PPR Panel WG addressed the following sub-question:

2. How can these criteria be applied and integrated into a tiered assessment strategy to determine whether dog studies should be retained or waived during the approval process of pesticide active substances?

To answer this sub-question, the PPR Panel WG developed proof-of-concept case studies to test the robustness of the tiered assessment strategy and to refine it where necessary.

3.2 | Data collection

The data sources used for this mandate are described below.

- In 2022, data were collected by EFSA (in the form of an Excel spreadsheet) as part of a retrospective analysis, leading to the publication of an editorial on the relevance of dog studies for the derivation of health-based guidance values for pesticide active substance approval (Panzarea et al., 2022). The data collected were then updated in 2024 (as part of an ISA call[8]) **(**Annex B.1**)** and cover EFSA Conclusions and European Commission Review Reports on the newly approved pesticide active substances and existing ones that underwent the (renewal) peer review process in the time range between January 2001 and November 2024.
- Seminal papers identified ad hoc by the PPR Panel WG.
- Other scientific and technical information, e.g. relevant EU/non-EU regulatory and governmental assessments.

3.3 | Data extraction

This section describes the methods used for the retrospective analysis performed. It was based on a comprehensive database of approved and non-approved active substances maintained by EFSA.

This initial database, created as part of the analysis conducted by the Chemicals Regulation Directorate, Health & Safety Executive in 2013 (Chemicals Regulation Directorate, Health, & Safety Executive, UK, 2013), has been updated with data from EFSA Conclusions and European Commission Review Reports on the newly active substances and existing ones that underwent the renewal review process up to November 2024 (Annex B.1).

The extracted data were compiled into an Excel spreadsheet and include the following: (1) general information on each pesticide active substance (i.e. name, CAS number, function, year of evaluation) and (2) information on the derivation of the HBGVs (i.e. ADI, ARfD, AOEL, AAOEL), including the type of study used to derive each HBGVs, the test species, justification for the overall uncertainty factor (UF) applied, route of exposure, critical endpoints, NOAEL/LOAEL values, target organs and other relevant toxicological data.

3.4 | Data interpretation and evaluation

Based on the Excel data set, pesticide active substances (hereafter referred as 'cases'), for which dog toxicity studies were used for the derivation of HBGVs, were identified and highlighted. In particular, comparative analyses were performed and data on lower NOAELs/LOAELs from available studies in other species from the data set were collected.

Although information on the four main types of HBGVs (ADI, AOEL, ARfD and AAOEL) was retrieved and collected, the PPR Panel WG agreed to focus the critical investigations of HBGVs in which dog toxicity studies were used to set ADIs and AOELs. The ARfDs and AAOELs were not analysed because the overall main objective of including dog toxicity studies in hazard assessment of pesticide active substances is to identify target organ(s) after repeated exposure rather than effects of a single (acute) exposure. In general, the derivation of ARfD and AAOEL relies on expert judgement on whether the critical effect selected for setting acute HBGVs is truly of an acute nature. The updated retrospective analysis showed that dog data were used in the derivation of ARfD and AAOEL in 36 and 12 cases, respectively. Most of the observed critical effects

[8]Individual Scientific Advisors (ISA) scheme supports EFSA's scientific work through the provision of expert advice.

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were related to general toxicity, gastrointestinal effects (vomiting and diarrhoea), neurological signs (excessive salivation and vomiting when recognised as signs of central nervous system toxicity), effects associated with methemoglobinaemia, blood ChE inhibition and local irritation, which are generally considered inadequate for extrapolation to humans for hazard characterisation. Moreover, in alignment with the 3R principle, it is neither advisable nor recommended to conduct dog toxicity studies solely for identifying acute effects. For the abovementioned reasons, the tiered assessment strategy (output of ToR 2) is not intended to be used for justifying the use of dog for evaluating acute toxicity, and the comparative analysis and critical investigation were focused on ADI and AOEL. For these two non-acute HBGVs, additional considerations and evaluations were made to determine whether the lowest NOAELs observed in dogs which were used as reference points for ADI or AOEL derivation reflected greater species-specific sensitivity or were rather a consequence of experimental design and/or biological variability.

The following approach was applied to exclude cases where the differences could be attributed to such variability:

1. If the ratio of $NOAEL_{other\ species}/NOAEL_{dog}$ is ≤ 3 (refer to Section 3.5 for justification for using a factor of 3), further analyses are not needed as the differences are likely due to experimental variability.
2. If the ratio of $NOAEL_{other\ species}/NOAEL_{dog} > 3$, but the $NOAEL_{other\ species} < LOAEL_{dog}$, further analyses are also not needed as the differences are likely due to dose spacing.
3. If the lower NOAEL in dog is based on a 1-year or 2-year toxicity study, the NOAEL from the 90-day dog study (when available) was retrieved. If the NOAEL from the 90-day dog study is in the same range (i.e. ratio of $NOAEL_{other\ species}/NOAEL_{90\text{-}d\ dog} \leq 3$) as the lower NOAEL from other species, no further analyses are needed as only the 90-day study in dog is required according to Regulation (EU) No. 283/2013.
4. The PPR Panel WG subsequently explored, as an alternative approach, whether the Human Equivalent Dose (HED) could serve as method to further elucidate interspecies NOAEL differences observed in the toxicological studies, which was calculated using body surface area (K_m factor) to translate and re-interpret critical NOAELs across species. The HED was calculated using the NOAELs of the different species and applying the equation mentioned in Section 3.5.

The HEDs calculated for the two species (dog and other species) were then compared. A similar approach to the NOAEL comparison was applied to the other metrics, e.g. comparing the ratio of $HED_{other\ species}/HED_{dog}$ and excluding cases where this ratio was equal to or less than 3.

Finally, in cases where interspecies differences in NOAELs or HEDs could not be attributed to experimental and biological variability, a critical assessment of the toxicological (mammalian) mode of action (MoA) of the active substance and potential dog-specific sensitivities was performed. As part of the investigation, the following questions were raised and answered, along with expert discussion to facilitate a critical evaluation and to draw well-supported conclusions on these specific cases.

- What factors can explain the different sensitivity between dogs and other species?
- Do dogs possess unique characteristics compared to other species that allow capture of specific hazards that otherwise would not be captured?
- Can ADME or comparative metabolism studies data explain the different sensitivity?
- What is the most plausible MoA of the active substance?

Benchmark dose (BMD) modelling was also considered to be applied in the retrospective analysis but can only be applied in cases where the variability in data allows sufficient confidence in the interpretation of the data. It is acknowledged that not all data sets in the retrospective analysis would allow for BMD modelling.

The assessment and overall conclusions were captured in the aforementioned Excel spreadsheet (Annex B.2).

3.5 | Metrics considered for data interpretation

Although dog toxicity studies have been commonly used as a basis for deriving HBGVs, a number of considerations must be taken into account to truly identify potential cases of dog-specific toxicity. To achieve this, a number of metrics and methods were employed by the WG (see **Section 3.4**) and described more in detail in this section.

Factor of 3

For the retrospective analysis, a factor of 3 between $NOAEL_{dog}$ and $NOAEL_{other\ species}$ was used as a screening threshold to account for the intrinsic experimental design limitations and biological variability from in vivo toxicological studies. The rationale behind this is that this factor is considered narrow, i.e. in the context of the dose-spacing intervals set in repeated dose toxicity studies (as the dose-spacing interval is often set between 3 and 10 in such studies); it also covers the standard toxicokinetic interspecies variability (i.e. a factor of 4.0; see EFSA Scientific Committee, 2012) and aligns with other retrospective analyses (Ono et al., 2018). This factor is not an uncertainty factor for risk characterisation but serves to distinguish differences attributable to study design (e.g. dose spacing, duration) or biological variation from true species-specific sensitivity.

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Dose spacing

A review and a comparison of the dosing regimens used in the toxicological studies were performed in order to evaluate whether the dose spacing could have influenced the selection of the lower NOAEL. If the NOAEL from another species was lower than the LOAEL in dogs, the observed difference was likely due to dose spacing rather than true species-specific sensitivity (refer to **Section 3.4**). Indeed, the dose levels used to define the NOAEL and LOAEL are discrete values established during the study design phase. The true LOAEL could lie anywhere between these tested doses, and the actual threshold for adverse effects may therefore overlap between the species.

Other metrics: HED

Allometric scaling is a well-known method used in regulatory toxicology that applies mathematical relationships between biological parameters, such as body weight (bw) and body surface area (BSA), to predict and support the comparison of doses and toxicokinetic parameters across species. Although HED is commonly used in pharmaceutical development, it is a general toxicological concept based on physiological scaling principles and is also recognised in regulatory toxicology for risk assessment of chemical substances, including pesticides (EFSA, 2023; Rhomberg & Lewandowski, 2004). The concept is based on the observation that metabolic rate and physiological parameters scale predictably with body size, following power laws. Scaling based on BSA is more closely correlated with physiological parameters, such as cardiac output and renal clearance, than scaling based on body weight alone (van Valkengoed et al., 2025). BSA can be calculated using the DuBois and DuBois formula (Burton, 2008):

$$\text{BSA} = 0.007184 \times (\text{Weight in kg})^{0.425} \times (\text{Height in cm})^{0.725}$$

A key component of this method is the K_m value,[9] which represents the ratio of BSA to bw and varies across species (Reagan-Shaw et al., 2008). It is used to convert the mg/kg dose used in a study to an mg/m^2 dose.

$$\text{Km} = \frac{\text{bw (kg)}}{\text{BSA}\left(\frac{\text{m}^2}{\text{kg}}\right)}$$

The K_m values are derived from average BSA calculations for both human and animal species. For instance, the BSA of a 70 kg human (assuming a height of 170 cm) is approximately 1.8 m^2, which corresponds to a K_m value of 38.7. For animals, the typical K_m values are approximately 3 for mice, 6 for rats, 12 for rabbits and 20 for dogs. The K_m value simplifies conversion to HED through the following formula (Reagan-Shaw et al., 2008):

$$\text{HED}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{Animal Dose}\left(\frac{\text{mg}}{\text{kg}}\right) \text{x} \frac{\text{Animal Km}}{\text{Human Km}}$$

Because dogs have a lower surface area-to-body mass ratio relative to rodents, dose extrapolations were normalised using allometric principles to facilitate the interpretation of apparent interspecies differences in NOAELs. In this assessment, such scaling (including the calculation of the HED) was used solely as a comparative and interpretative screening tool and not for the derivation of HBGVs. The application of standard regulatory uncertainty factors remains unchanged and fully protective of human health.

BMD modelling

The consideration of using and comparing BMD values between species is driven by the fact that the identification of the NOAEL is highly dependent on the selection of spacing between the tested doses in the toxicological studies (Niemann et al., 2023). Since the NOAEL is defined as the highest dose tested in a study without evidence of an adverse effect, higher NOAELs are often observed when the statistical power of the study is low (e.g. small group size). In contrast, the BMD approach evaluates the full dose–response relationship, allowing for a more refined and probabilistic analysis of the critical effect. It also allows estimation of the probability of response at any dose level.

The BMD represents a dose level associated with a predefined change in response relative to the background response that can be reliably measured, the so-called benchmark response (BMR). The BMDL is the lower bound of the BMD's credible interval and this value is normally used as a reference point. The concept of BMD modelling offers a more refined analysis of toxicological data and has been described in further detail in several publications, including an EFSA guidance on the use of the BMD approach in risk assessment (Haber et al., 2018; EFSA Scientific Committee, 2022). Nevertheless, in the context of pesticide risk assessment, the NOAEL is the reference point most often used to derive HBGVs (and also required in Commission Regulation (EU) No. 283/2013).

[9]In the context of toxicology and HED, the term K_m has no relation to the Km used in enzyme kinetics (Michaelis–Menten constant). Even though they share the same symbol (K_m), they represent entirely different concepts in different scientific domains. The similarity in notation is purely coincidental. K_m is a species-specific factor used in allometric scaling to convert doses between animals and humans based on differences in body surface area (BSA) relative to body weight (bw). It is defined as: K_m = body weight (kg)/body surface area (m^2).

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3.6 | Basis for the development of the tiered assessment strategy

The tiered assessment strategy was developed in response to findings from the critical investigations of cases, where experimental and biological variability could not adequately explain the differences in NOAELs or HEDs across species. These unexplained differences highlighted the need for a structured and scientifically robust approach to assess interspecies differences in toxicological hazard assessment. A central motivation for developing the strategy was the need to systematically address cases where dogs appeared to show greater sensitivity to target organ toxicity. This led to the identification of the specific data required to evaluate whether such sensitivity was truly species-specific or instead attributable to other factors such as study design, dose spacing or toxicokinetics. The resulting tiered assessment strategy provides a set of proposed methods for evaluating higher sensitivity of dogs, together with clearly defined data requirements necessary to reach scientifically sound conclusions.

The PPR Panel WG first considered several known scientific factors, such as species-specific differences in ADME processes and physiological characteristics that may contribute to the increased dogs' sensitivity to exposure to certain pesticides. In particular, the type and quality of information required to enable a scientifically reliable comparison of these factors across different species was discussed, and such information ultimately served as the conceptual and data-driven basis for developing the tiered assessment strategy.

Next, the potential strategies to mitigate the uncertainties and/or resolve data gaps identified during the case investigations were addressed. In some cases, the critical investigations precluded conclusive interpretations due to the presence of unspecific effects (e.g. reduced body weight or body weight gain) and/or limited scientific understanding on the toxicological mode of action for certain active substances. Consequently, the PPR Panel WG discussed not only the type of information required to resolve these uncertainties and fill the data gaps but also explored alternative or other (new) approaches for handling situations where uncertainties/data gaps could not be fully eliminated. Finally, the tiered assessment strategy was drafted based on these foundational considerations and envisioned a stepwise process grounded in scientific evidence to justify whether dog toxicity studies are necessary on a case-by-case basis. The strategy is designed to ensure that decisions are based on clearly defined criteria and supported by relevant, high-quality data.

4 | ASSESSMENT

4.1 | Outcome of the updated retrospective analysis (ToR 1)

Among the total of 461 active substances with published EFSA Conclusions and European Commission Review Reports (up to November 2024), dog toxicity studies were found to be relevant for the setting of ADI and AOEL in 107 (23%) and 183 cases (40%), respectively (see Figure 1).

Following the successive screening phase to identify and exclude cases for which the differences between the NOAEL in dog and the next lower NOAEL in other species were a consequence of experimental design and/or biological variability, only three cases for ADI (cyclaniliprole, methiocarb and sodium 5-nitroguaiacolate) and nine cases for AOEL (amidosulfuron, cyclaniliprole, florasulam, fluoxastrobin, imazaquin, iprovalicarb, meptyldinocap, myclobutanil and sodium 5-nitroguaiacolate) remained open for further analysis (see Figure 1 and Annex B.2 for further details).

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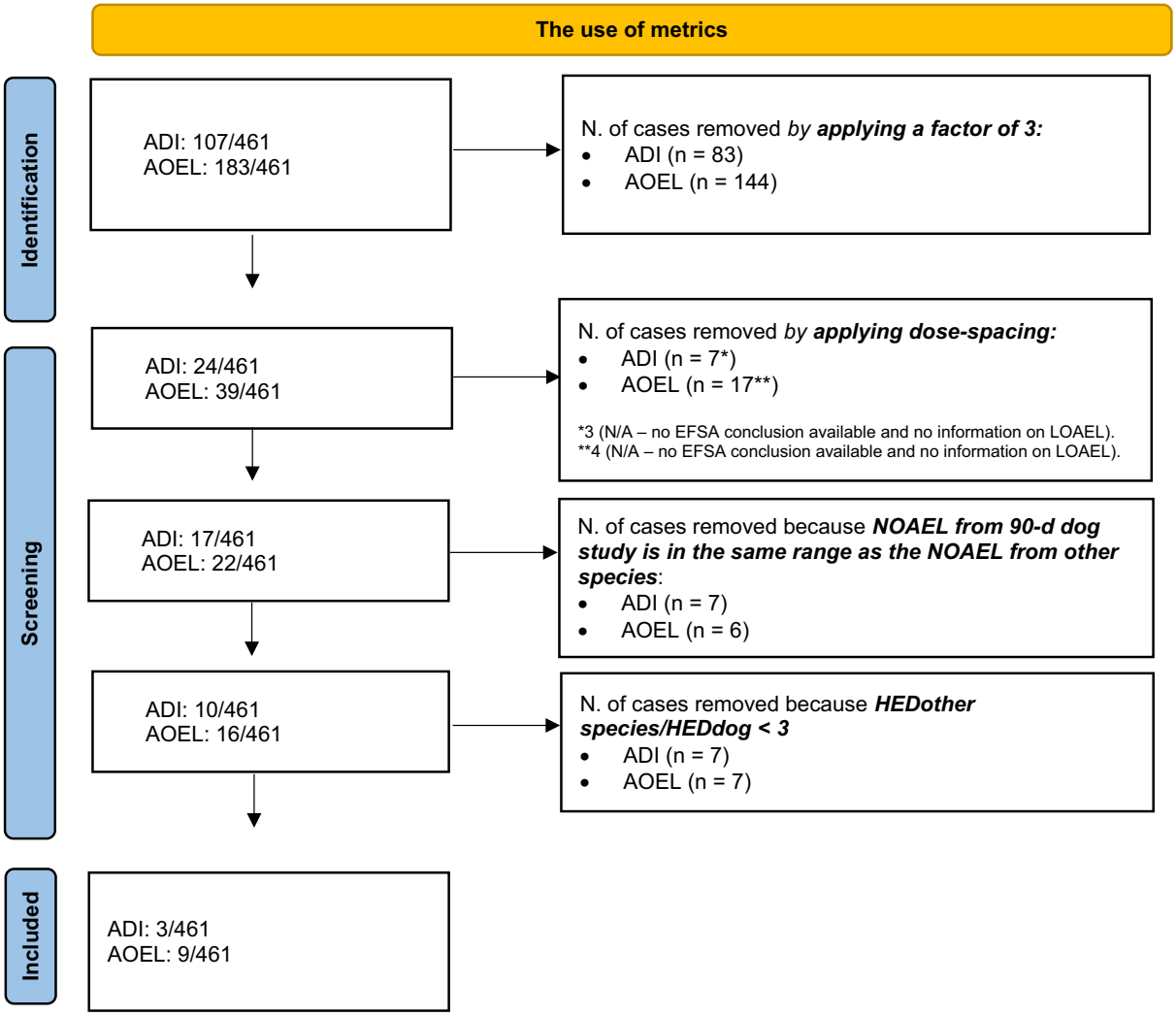


FIGURE 1 Retrospective analysis of the relevance of regulatory accepted 90-day and 1-year repeated-dose oral toxicity studies in dogs for the derivation of ADI and AOEL values of 461 active substances.

4.1.1 | Common observations and patterns of toxicity identified

Of the 12 cases included for further analysis (3 for ADI and 9 for AOEL), the setting of the NOAEL in the 90-day dog studies was mainly based on unspecific effects. These cases were related to general physiological responses, such as changes in body weight or body weight gain, and did not indicate any target organ toxicity or a toxicological mode of action but rather tolerability limitations associated with high dose administration (see Appendix B). Unspecific effects arise from general physiological or cellular disruption, such as oxidative stress, energy depletion or inflammation, and typically appear across species at higher doses because they are not driven by a particular molecular target. In contrast, specific effects result from defined interactions with particular biological targets (e.g. receptors, enzymes, ion channels), often occurring at lower doses and showing clearer species differences due to variations in target structure and function. In the cases where only unspecific effects were identified, the PPR Panel WG highlighted that the absence of toxicokinetic data in dogs precludes an assessment of whether dogs show any unique species-specific features relative to other test species.

In three cases only (methiocarb, imazaquin, florasulam), the effects observed in dogs were on specific organs (methiocarb: reduction in erythrocyte and retinal cholinesterase; imazaquin: anaemia and skeletal myopathy; florasulam; anaemia, increased liver weight, and adrenal and liver vacuolation) and possibly associated with a specific mechanism of action of the active substance. Of these three, only florasulam is currently approved in the EU.

Although not identified in the retrospective analysis as substances where dog studies were critical for hazard characterisation, for some pesticide active substance classes, some recurrent patterns of toxicity were identified, namely:

- In the case of imazaquin, anaemia and skeletal myopathy were observed in 1-year dog toxicity studies, and it seemed that dog was the only species in the data set able to predict this hazard. It is noteworthy that similar effects, i.e. anaemia and/or skeletal myopathies, were also reported after 1-year exposure to other **imidazolinone herbicides** such as imazamethabenz-methyl, imazapic and imazethapyr.

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- In the cases of phenoxyacetic acids (e.g. 2,4-D and 2,4-DB) and pyridyloxyacetic-acid (e.g. triclopyr) herbicides, lower NOAELs were consistently observed in dog repeated dose studies compared to respective studies in other species. These NOAELs were based on kidney toxicity and a possible MoA was related to the fact that dogs have lower capacity to actively secrete organic acids via the renal organic acid secretory system compared to rats, allowing organic acids to accumulate in the proximal renal tubular cells. The human relevance of such an effect remains unclear.
- Dog may be more sensitive to the effects of **macrocyclic lactones** (e.g. milbemectin). One study suggests that dogs of certain breeds are more sensitive to this class of compounds due to mutations in the *ABCB1* gene. This mutation results in a non-functional P-glycoprotein (P-gp), which in turn eventually leads to the accumulation of P-gp substrates in the brain and consequent neurotoxic symptoms (Merola & Eubig, 2012).
- Lower NOAELs from dog studies were also consistently observed with **anthranilic diamide insecticides** (e.g. chlorantraniliprole, cyantraniliprole, cyclaniliprole flubendiamide) based on similar types of effects involving the liver as a key target organ.
- Dogs seem to be more sensitive to the acute effects associated with methaemoglobin formation. Congenital conditions could impact the readout and interpretation of toxicological studies, introducing a species-specific bias and making the test system not relevant for human risk assessment. An example of this is, in fact, methemoglobinaemia. This congenital condition is known in certain breeds, including Beagle dogs (Srivastava et al., 2002).

Details of the PPR Panel WG assessment of the analysis above are reported in Annex B.2.

In addition, the PPR Panel WG performed an evaluation of the ECHA's existing Classification, Labelling and Packaging (CLP) Regulation's database[10] in order to explore the number of incidences of which the dog was used as the only species for the classification of pesticide active substances as STOT RE (single target organ toxicity after repeated exposure). A total of 84 pesticidal active substances have been listed in Annex VI of Regulation (EC) No 1272/2008 (up to ATP 22[11]) harmonised classification of STOT RE, among which 40 substances have a Risk Assessment Committee (RAC) Opinion that is publicly available. For these 40 substances, seven (18%) revealed organ-specific effects only observed in dogs, namely flupyradifurone, ipconazole, metosulam, picolinafen, spiroxamine, spirodiclofen and tebufenpyrad.

The eye was the most frequently affected organ (3 substances: ipconazole, metosulam, spiroxamine). For metosulam, notably higher concentrations in dog eyes compared with rodents suggest that the findings are likely driven by toxicokinetic differences rather than by a dog-specific toxicodynamic mechanism. Effects on muscle, skin, thyroid, blood, liver and the gastrointestinal tract each occurred only once and each for different substances, and no investigation of specific mechanism of action was identified in any of the RAC opinions.

For two substances (flupyradifurone and tebufenpyrad), the ADI and AOEL values established during EFSA peer review were based on rat studies, indicating that dogs were not the most sensitive species. For the remaining five substances, HBGVs were derived from dog NOAELs; however, the observed interspecies differences can be explained by normal experimental and biological variability, dose spacing, or allometric scaling, rather than by genuine dog-specific sensitivity (for details on applied metrics please see Chapter 3.5).

Overall, no evidence of a dog-specific mechanism of action was identified. Notably, although the mechanism underlying the observed toxicity in dogs remains unclear, this does not affect the overall hazard characterisation of these substances.

The details of the PPR Panel WG assessment on the harmonised STOT RE-classified substances are reported in Annex B.3.

4.1.2 | Application of the metrics

Factor of 3 and dose spacing

For ADI derivation, the NOAEL was selected as the reference point from dog toxicity studies in 107 cases. In 83 of these cases, a factor of ≤ 3 could explain the difference in NOAELs between dog and the other species. Then, among the remaining 24 cases, dose spacing could account for the difference in NOAELs in four cases (Table 3). For three cases, no EFSA conclusion was available, and it could not be evaluated whether $NOAEL_{other\ species} < LOAEL_{dog}$. It is of note that a ratio close to 0 means that the NOAEL in other species is lower than the one in dog, and that the data set of studies includes at least one NOAEL in other species that is lower than dog but not used for the derivation of the HBGVs.

[10] https://chem.echa.europa.eu/obligation-lists/clhList?pageIndex=1&pageSize=100.

[11] ATP (Adaptation to Technical Progress): regulatory updates adopted by the European Commission to amend harmonised classification and labelling; the present list includes ATPs up to and including ATP 22.

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TABLE 3 Summary of the outcome of the retrospective analysis with regard to the ratio $NOAEL_{other\ species}/NOAEL_{dog}$ and dose spacing for the ADI derivation.

Ratio: $NOAEL_{other\ species}/NOAEL_{dog}$	Number of cases	Dose spacing: $NOAEL_{other\ species} < LOAEL_{dog}$?
$0 \le x < 1^{\&}$	2	–
$1 \le x < 2$	49	–
$2 \le x < 3$	19	–
$3 \le x < 4$	13	–
$4 \le x \le 10$	21	**Y: 4/21** **N: 14/21** N/A*: 3/21
$10 < x < 50$	1	**N: 1/1**
≥ 50	2	**N: 2/2**

*N/A: EFSA conclusion not available, information is not enough to evaluate whether $NOAEL_{other\ species} < LOAEL_{dog}$. N: NO, Y: YES.
&Values < 0.5 were rounded to 0. Values ≥ 0.5 were rounded to 1.

For AOEL derivation, the NOAEL was selected as the reference point from dog toxicity studies in 183 cases. In 144 of these cases, a factor of ≤ 3 could explain the difference in NOAELs between dog and other species. Among the remaining 39 cases, dose spacing could account for the difference in NOAELs in 13 cases (Table 4). For four cases, no EFSA conclusion was available, and it could not be evaluated whether $NOAEL_{other\ species} < LOAEL_{dog}$.

TABLE 4 Summary of the outcome of the retrospective analysis with regard to the ratio $NOAEL_{other\ species}/NOAEL_{dog}$ and dose spacing for the AOEL derivation.

Ratio: $NOAEL_{other\ species}/NOAEL_{dog}$	Number of cases	Dose spacing: $NOAEL_{other\ species} < LOAEL_{dog}$?
$0 \le x < 1^{\$}$	11	–
$1 \le x < 2$	82	–
$2 \le x < 3$	34	–
$3 \le x < 4$	17	–
$4 \le x \le 10$	32	**Y: 13/32** **N: 16/32** N/A*: 3/32
$10 < x < 50$	6	**N: 5/6** N/A*: 1/6
$x \ge 50$	1	**N: 1/1**

*N/A: EFSA conclusion not available, information is not enough to evaluate whether $NOAEL_{other\ species} < LOAEL_{dog}$. N: NO, Y: YES.
$Values < 0.5 were rounded to 0. Values ≥ 0.5 were rounded to 1.

In seven cases, the ADI was based on a 1- or 2-year dog toxicity study with at the same time the NOAEL from the 90-day dog study being in the same range as the lower NOAEL from other species (i.e. ratio of $NOAEL_{other\ species}/NOAEL_{90\text{-}d\ dog} \le 3$), with the same being true for the AOEL in six cases.

Human Equivalent Dose (HED)

Scaling factors may play a relevant role in the lower NOAEL observed in dogs as compared to that observed in rats. Dogs have a lower surface area-to-body mass ratio relative to rodents, which can influence systemic exposure when doses are expressed on a mg/kg bw basis. To support interpretation of apparent interspecies differences, HED was therefore explored as a comparative screening metric.

First, for each case not excluded after the consideration of the aforementioned metrics, the critical NOAELs observed for the second species and for dogs were normalised to body surface area as described in Section 3.5 using the K_m value reported in the United States Food and Drug Administration (FDA) guidance (FDA, 2005). NOAELs expressed by body mass were also reported for these cases (Tables 5 and 6). Second, the corresponding HED was calculated accordingly, using the conversion factors reported in this FDA guidance.

A ratio $HED_{other\ species}/HED_{dog}$ within the range of threefold was considered indicative of no meaningful difference, assuming that the standard UF of 100 (comprising 10 for interspecies and 10 for intraspecies variability) remains fully applicable and protective of human health (Tables 5 and 6).

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TABLE 5 NOAEL expressed as body mass and HED calculation – ADI. Marked boxes = cases for which $HED_{other\ species}/HED_{dog}$ is above 3.

Active substance	NOAEL expressed as BODY MASS [mg/m^2] HED CALCULATION [mg/kg bw per day]	$HED_{other\ species}/HED_{dog}$
Amidosulfuron	Dog: 66.4 × 20 = 1328 Rat: 495.42 × 6 = 2972 HED (dog): 66.4/1.8 = 36.8 HED (rat): 495.42/6.2 = 79.9	2
Amitraz	Dog: 0.25 × 20 = 5 Rat: 1.6 × 6 = 9.6 HED (dog): 0.25/1.8 = 0.13 HED (rat): 1.6/6.2 = 0.25	2
Cyantraniliprole	Dog: 1 × 20 = 20 Rat: 7 × 6 = 42 HED (dog): 1/1.8 = 0.5 HED (rat): 7/6.2 = 1.13	2
Cyclaniliprole	Dog: 1.29 × 20 = 25.8 Rat: 249 × 6 = 1494 HED (dog): 1.29/1.8 = 0.7 HED (rat): 249/6.2 = 40.2	**57**
Fluoxastrobin	Dog: 1.5 × 20 = 30 Rat: 9 × 6 = 54 HED (dog): 1.5/1.8 = 0.8 HED (rat): 9/6.2 = 1.45	2
Formetanate	Dog: 0.4 × 20 = 8 Rat: 2.3 × 6 = 13.8 HED (dog): 0.4/1.8 = 0.22 HED (rat): 2.3/6.2 = 0.37	2
Imazaquin	Dog: 25 × 20 = 500 Mouse: 150 × 3 = 450 HED (dog): 25/1.8 = 13.8 HED (mouse): 150/12.3 = 12.2	1
Methiocarb	Dog: 0.25 × 20 = 5 Rat: 4.3 × 6 = 25.8 HED (dog): 0.25/1.8 = 0.14 HED (rat): 4.3/6.2 = 0.69	**5**
Quinmerac	Dog: 7.9 × 20 = 158 Mouse: 31 × 3 = 93 HED (dog): 7.9/1.8 = 4.4 HED (mouse): 31/12.3 = 2.5	1
Sodium 5-nitroguaiacolate	Dog: 0.29 × 20 = 5.8 Mouse: 39.6 × 6 = 237.6 HED (dog): 0.29/1.8 = 0.16 HED (mouse): 39.6/6.2 = 6.38	**40**

TABLE 6 NOAEL expressed as body mass and HED calculation - AOEL. Marked boxes = cases for which $HED_{other\ species}/HED_{dog}$ is above 3.

Active substance	NOAEL expressed as BODY MASS [mg/m^2] HED CALCULATION [mg/kg bw per day]	$HED_{other\ species}/HED_{dog}$
Amidosulfuron	Dog: 66.4 × 20 = 1328 Rat: 792 × 6 = 4776 HED (dog): 66.4/1.8 = 36.8 HED (rat): 792/6.2 = 127.7	**4**
Clomazone	Dog: 13.3 × 20 = 266 Rat: 84 × 6 = 594 HED (dog): 13.3/1.8 = 7.4 HED (rat): 84/6.2 = 13.5	2
Cyclaniliprole	Dog: 1.29 × 20 = 25.8 Rat: 1023 × 6 = 6139 HED (dog): 1.29/1.8 = 0.71 HED (rat): 1023/6.2 = 165	**232**
Ethephon	Dog: 2 × 20 = 40 Rat: 14 × 6 = 84 HED (dog): 2/1.8 = 1.11 HED (rat): 14/6.2 = 2.25	2

(Continues)

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TABLE 6 (Continued)

Active substance	NOAEL expressed as BODY MASS [mg/m^2] HED CALCULATION [mg/kg bw per day]	HED$_{other\ species}$/ HED$_{dog}$
Florasulam	Dog: 5 × 20 = 100 Rat: 100 × 6 = 600 HED (dog): 5/1.8 = 2.7 HED (rat): 100 /6.2 = 16.1	**6**
Fluoxastrobin	Dog: 1.7 × 20 = 34 Rabbit: 30 × 25 = 750 HED (dog): 1.7/1.8 = 0.94 HED (rabbit): 30/3.1 = 9.6	**10**
Imazaquin	Dog: 25 × 20 = 500 Rabbit: 250 × 25 = 6250 HED (dog): 25/1.8 = 13.8 HED (rabbit): 250/3.1 = 80.64	**6**
Iprovalicarb	Dog: 3 × 20 = 60 Rat: 146.3 × 6 = 877.8 HED (dog): 3/1.8 = 1.6 HED (rat): 146.3 /6.2 = 23.6	**15**
Meptyldinocap	Dog: 1.63 × 20 = 32.6 Rabbit: 12 × 12 = 144 HED (dog): 1.63/1.8 = 0.90 HED (rabbit): 146.3 /3.1 = 47.2	**52**
Milbemectin	Dog: 3 × 20 = 60 Rat: 12.4 × 6 = 74.4 HED (dog): 3/1.8 = 1.6 HED (rat): 12.4 /6.2 = 2	1
Myclobutanil	Dog: 0.34 × 20 = 6.8 Rat: 16 × 6 = 96 mg/m^2 HED (dog): 0.34/1.8 = 0.2 HED (rat): 16 /6.2 = 2.6	**13**
Penconazole	Dog: 3 × 20 = 60 Rat: 25 × 6 = 150 HED (dog): 3/1.8 = 1.7 HED (rat): 25/6.2 = 4	2
Quinmerac	Dog: 7.9 × 20 = 158 Rabbit: 30 × 12 = 360 HED (dog): 7.9/1.8 = 4.4 HED (rabbit): 30/3.1 = 9.7	2
Sodium 5-nitroguaiacolate	Dog: 0.7 × 20 = 14 Rabbit: 13.6 × 12 = 163.2 HED (dog): 0.7/1.8 = 0.4 HED (rabbit): 13.6 /3.1 = 4.3	**11**
Spinetoram (XDE-175)	Dog: 2.5 × 20 = 50 Mouse: 9 × 3 = 27 HED (dog): 2.5/1.8 = 1.4 HED (mouse): 9/12.3 = 0.7	1
Triclopyr	Dog: 0.5 × 20 = 10 Rat: 5 × 6 = 30 HED (dog): 0.5/1.8 = 0.3 HED (rat): 5/6.2 = 0.8	3

Benchmark Dose (BMD) Level

The PPR Panel WG used BMD modelling to derive a BMDL on a selected case study to investigate whether the use of the BMD approach would deliver different information than the NOAEL and would result in a different interpretation of the reference point.

The selected substance was **cyantraniliprole** based on the target organ toxicity, i.e. the same between dog and rat, and effect, i.e. increase in relative liver weight, for which a specific BMR of 15% is already established and agreed in the scientific community (EFSA, 2025).

The results of the BMD modelling are reported in Tables 7 and 8 for dog and rat, respectively.

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TABLE 7 Model average BMD_{15}: 90-day dog – relative liver weight.

Group	BMDL	BMD	BMDU
M	0.413	1.187	3.033
F	1.686	8.728	30.956

Note: BMDU/BMDL < 20 (M, F).

TABLE 8 Model average BMD_{15}: 90-day rat – relative liver weight.

Group	BMDL	BMD	BMDU
M	951.915	1366.050	1960.854
F	21.632	99.058	449.923

Note: BMDU/BMDL < 7 (M, F).

The BMD analysis did not reveal any new information compared to the NOAEL setting, i.e. dog was more sensitive species for relative liver weight with CES/BMR of 15%. In case of NOAEL setting, the ratio of $NOAEL_{rat}/NOAEL_{dog}$ was 7.

The WG noted that the applicability of BMD analysis is limited in this work as it requires robust experimental design and access to raw data, which was not always possible for the cases evaluated in the retrospective analysis.

4.1.3 | Uncertainties pertaining to retrospective analysis

As part of the retrospective analysis evaluation phase, several uncertainties (see below) were identified. They were mainly related to limitations in the data provided within study dossiers and restricted an extensive evaluation of the species sensitivity:

- **Incomplete toxicokinetic information**. The primary goals of OECD TG 417 (OECD, 2010) are to describe the mass balance of the active substance (in urine, faeces, tissues, etc.) and to detect major metabolites (> 5%). However, other toxicokinetic characteristics and detailed metabolite profiles often remain undefined due to the highly variable quality of studies, differences in study design, variable and sometimes outdated analytical methods, missing data and inadequate reporting.
- **Limited use of comparative in vitro metabolism (CIVM) studies**. CIVM studies have been inconsistently employed in other species (besides rat and human), leading to uncertainty in the interpretation of metabolic differences across species.
- **Toxicodynamic differences**. Similar to toxicokinetic studies, toxicodynamic understanding is hampered by the highly variable quality of studies, missing data and inadequate reporting.
- **Expert judgement**. The evaluation of studies as well as their selection for the derivation of HBGVs relies on an expert-based peer-review process, which is highly dependent on the experience of the experts.

4.1.4 | Identification of criteria for determining the inclusion of dog toxicity studies for active substance approval process

Following the critical investigation on the 12 specific cases (for both ADI and AOEL), the PPR Panel WG elaborated criteria to robustly determine whether and under which conditions dog toxicity studies may be waived for the approval process of pesticide active substances, addressing the assessment question posed during problem formulation ('*What are the criteria to determine whether dog toxicity studies should be included as part of the data requirement for the approval of active substances included in plant protection products?*'). It is important to note that, while setting the criteria, the PPR Panel WG considered the data requirements currently set out in Commission Regulation (EU) No. 283/2013 for pesticide active substances.

First and foremost, to allow a clear and robust assessment of short-term toxicity (and potentially of other toxicological endpoints) without the inclusion of dog toxicity studies, it is critical that a comprehensive toxicological data set in one species (namely rat) is available, in line with the current data requirements listed in the Commission Regulation (EU) 283/2013, e.g. including ADME, in vitro and in vivo toxicokinetic data on rat (or on other rodent species like mouse, if justified). In addition, if available, it is relevant to retrieve information and gain understanding on the MoA (pesticidal and toxicological) of the substance (and potentially substances belonging to the same chemical class) to consider its toxicodynamics, as this information may provide sufficient justification to waive dog toxicity studies (see Section 4.2.1 for further elaboration).

For nine out of the 12 cases identified in the retrospective analysis, unspecific effects in dog toxicity studies were selected as the critical effects for deriving ADIs or AOELs. In the remaining three cases, i.e. methiocarb, florasulam and imazaquin, the effects observed in dogs involved specific target organs and were possibly associated with a specific mechanism of action of the active substance. These cases were documented and taken into account in the assessment strategy flowchart. A data deficiency consistently observed in the critical investigation that precluded complete evaluation of the cases was the lack of reliable ADME or toxicokinetic (TK) data across species. Although the retrospective analysis cannot fully exclude the possibility of a dog-specific target organ toxicity, it reinforced the importance of a robust ADME strategy to validate and justify the relevance of selecting the rat (or mouse, if justified) as the suitable species for the hazard assessment. In

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particular, a CIVM study using dog, rat (or mouse, if justified) and human hepatocytes (microsomes may be used on a case-by-case basis) could facilitate a scientifically robust comparison of ADME properties among species.

Finally, understanding the internal exposure to an active substance across species, especially in dogs, rats (or mice, if justified) and humans, could further provide justification for potentially excluding dogs from toxicity testing. For example, if rats were shown to have a higher body burden or slower excretion of the active substance than dogs, this would support the rat as a more adequate test system to detect and quantify hazards for the substance of interest, thus justifying the waiving of dog toxicity studies. Emerging methodologies in hazard assessment, such as PBK modelling, could be employed to simulate and compare the internal exposure across species. Obtaining comprehensive ADME information in multiple species would be essential to appropriately generate the models to accurately predict internal exposure levels in different species. Consequently, this information supports the assessment of the added value of including the dog as a second species or, alternatively, supports the quantification of the level of uncertainty when a dog study is absent. The minimal information required for such PBK modelling could be obtained using various in vitro and in silico methods (refer to the Table in Section 4.2.1 for more details).

Overall, these three aspects (i.e. a comprehensive toxicological data set in rats, a proper ADME strategy and an understanding of the internal exposure across species) served as the foundation for establishing the minimum information required for applying the tiered assessment strategy described further in the section below.

Appendix A provides further scientific considerations and details on the elements of ADME and TK that served for the inclusion in the assessment strategy.

4.2 | Development of a tiered assessment strategy (ToR 2)

A tiered assessment strategy for justifying the inclusion or exclusion of the dog as a second species for evaluating repeated-dose toxicity of a pesticide active substance was conceived considering the three main criteria (i.e. a comprehensive toxicological data set in rats, a proper ADME strategy, and an understanding of the internal exposure across species) and the sub-question of the problem formulation for ToR2 ('*How can these criteria be applied and integrated into a tiered assessment strategy to determine whether dog studies should be retained or waived during the approval process of pesticide active substances?*').

To develop the strategy, the PPR Panel WG first identified the minimum information required (Table 9) and then specified acceptable test types, applicable OECD guidelines, and other published protocols for conducting the strategy (see **Section 4.2.1**). Based on the established information requirement, a workflow was created to evaluate different possible outcomes, also depending on the quality, reliability and completeness of available data. The PPR Panel WG acknowledged that there are a few outcomes in which waiving of dog toxicity studies could be scientifically and readily justified without running the entire strategy and thus established an initial step ('preliminary phase 1') to determine whether a (new) active substance would meet one of these outcomes and allow an early 'exit' from the assessment strategy. Next, in order to substantiate the scientific justification of waiving dog toxicity studies, it is pertinent to demonstrate that rat (or mouse, if justified) is a suitable model for hazard assessment; thus, this substantiation forms another early step ('preliminary phase 2') before entering the full flowchart of comparing data and information, including PBK modelling predictions and/or in vivo TK data, between rats (or mice, if justified) and dogs.

Finally, to test the feasibility of employing this assessment strategy, proof-of-concept case studies were developed by the PPR Panel WG using the currently available information (i.e. existing evidence) for the selected substances.

4.2.1 | Development of the tiered assessment strategy

Following the collection of data on the active substances, the workflow reported in Figure 2 was designed to evaluate the information in a prioritised sequence. This workflow serves to determine and justify the selection of the species to adequately identify and characterise hazards to human health. This assessment strategy is primarily developed for new active substances (NAS) to be placed on the EU market as an approach to determine whether dog toxicity testing is truly necessary for the hazard identification and characterisation of the NAS. The proposed strategy is meant to guide applicants and risk assessors to provide information to support the waiving of the dog as a second species for the assessment of pesticide active substances. In this context, additional information not included in the strategy, but supporting the waiving decision, can be considered.

It should be specified that the tiered nature of the assessment strategy refers to a series of steps (see paragraph below for details) to be followed in sequence, and, depending on the outcome of each step, the next suitable course of action is taken, eventually leading to the final conclusion that would determine if a dog toxicity study is truly necessary for the hazard assessment.

The assessment strategy is divided into three main parts:

- Gathering available data
- Running preliminary phases 1 and 2
- Applying the flowchart

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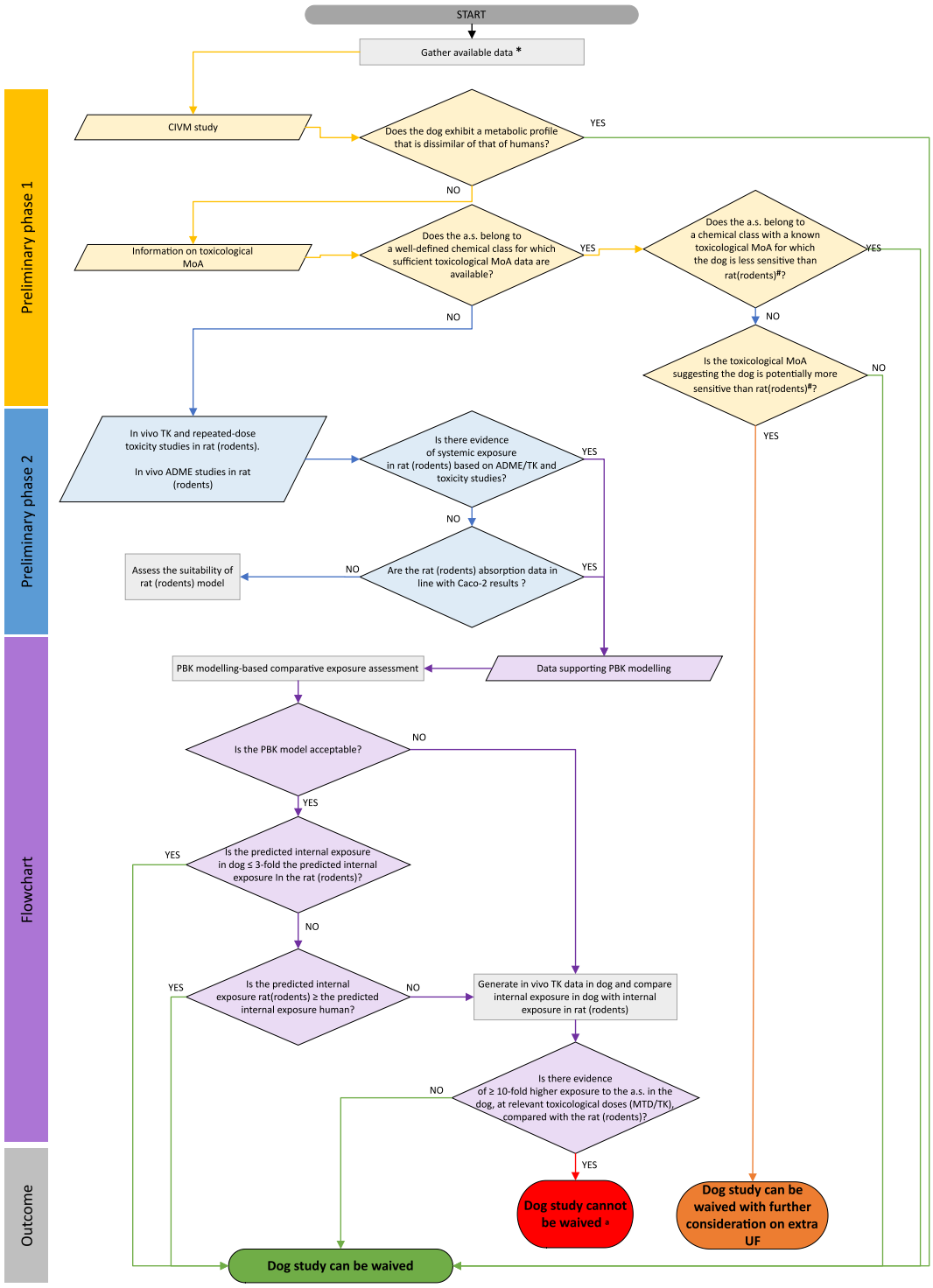


FIGURE 2 Assessment strategy. This figure outlines the assessment strategy used to determine whether a 90-day dog toxicity study provides additional scientific value for human health hazard assessment. The approach integrates mechanistic toxicodynamic (TD) with quantitative toxicokinetic (TK) information and PBK modelling, and is organised into three sequential components, each with defined exit points that may scientifically justify the waiving of the dog toxicity study. The first component (**preliminary phase 1**) evaluates species relevance. A comparative in vitro metabolism (CIVM) study in dog, rat (or mouse, if justified) and human hepatocytes is assessed to determine whether the dog produces a similar metabolic profile as humans. A dissimilar profile indicates that the dog is not fit-for-purpose. This phase also considers the toxicological MoA: if existing evidence shows that rodents are more sensitive than dogs (or, conversely, that dogs are more sensitive, but with quantifiable uncertainty), the dog toxicity study may be waived. If the MoA information is insufficient, the assessment moves to the next phase. **Preliminary phase 2** examines whether the rat (or mouse, if justified) provides adequate systemic exposure and can serve as a suitable species for hazard assessment. ADME, TK and toxicity data are used to confirm exposure at relevant dose levels. If there is limited internal exposure in rats, human intestinal permeability data (from Caco-2 cells) are compared with rat (or mouse) oral absorption data to evaluate concordance before continuing the assessment. If the rat (or mouse) is considered suitable, the strategy advances to the main flowchart, where PBK modelling is used to quantitatively compare internal exposure (C_{max} and AUC) across species. Model evaluation in rats (or mice) ensures predictive reliability, followed by comparisons between dog and rat (or mouse), and between rat (or mouse) and human. If needed, in vivo dog TK data are generated; a threshold of more than 10-fold higher internal exposure in dogs indicates that a dog toxicity study may be warranted.
[a]Alternatively, the regulatory approach could also consider the applicability of an additional uncertainty factor (UF), the magnitude of which would be determined on a case-by-case basis, depending on the available data and context of the assessment.
*Further information is reported in Section 4.2.1.1 (Table 9) of the Scientific Opinion.
#Further information is reported in Section 4.2.1.2 Running preliminary phases 1 and 2 of the Scientific Opinion.

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4.2.1.1 | *Gathering available data*

As part of the assessment strategy proposed in this Scientific Opinion, once the available information and data on the new active substance have been generated as part of the approval process of pesticide active substances, they should be categorised as shown in Table 9.

However, following the tiered nature of the assessment strategy (Figure 2), preliminary phase 1 puts emphasis first on the evaluation of comparative in vitro metabolism (CIVM) data, followed by an assessment of the existing information on the toxicological MoA. This initial step aims to identify early outcomes, under which the waiving of dog toxicity studies could be scientifically justified without progressing through the main flowchart and without generating the complete data set of studies outlined in Table 9. Subsequently, preliminary phase 2 prioritises the investigation of ADME and toxicokinetic data in rats (or mice, if justified) to substantiate the suitability of the rat (or mouse) as a model for hazard assessment.

Then, to inform the main flowchart, a minimum data set to support the development and evaluation of a PBK model is needed. Further considerations on PBK model parameterisation are included in a recently published PPR Panel Scientific Opinion on QIVIVE of DNT IVB data (EFSA PPR Panel, 2025) and also in OECD Guidance Document GD No. 331 (OECD, 2021) that supports the development and evaluation of PBK models to increase confidence in their regulatory application.

TABLE 9 Gathering available data.

Type of data	Description
Information on NAS	
• Chemical class • Pesticidal Mode of Action (pMoA) • Toxicological MoA	Some NAS may belong to a known chemical class of compounds sharing the same pMoA. It is of note that in some cases, compounds sharing the same pMoA may also share the same toxicological MoA (i.e. toxicity). Read-across, QSAR tools and/or in silico predictions could be supportive information in this context. Consideration of the prior knowledge of toxicological MoA (Physiological-anatomical characteristics of the dog; Toxicophore). Available information from EFSA retrospective analysis may be used.
Physicochemical properties	
• Molecular weight (MW) • Solubility (different pHs and media) • Partition coefficient n-octanol/water (Log Pow) • Volatility • pKa (dissociation constant in water)	Adequate physicochemical characterisation of the active substance can provide information about the relationship between its chemical structure and ADME properties. Moreover, physicochemical characterisation is needed to perform in vitro assays. This information is already included as part of the Data Requirements listed in Reg. 283/2013 and internationally agreed methodologies are already available to generate those data (rf. Document 52013XC0403(02)[12]), namely: - Water solubility (OECD TG 105; OECD 1995a), solubility in organic solvent (CIPAC Method MT 181). Partition coefficient n-octanol/water (OECD TG 107; OECD 1995b) - Vapour pressure, volatility (OECD TG 104; OECD, 2006) - Dissociation constants in water (OECD TG 112; OECD, 1981)
ADME parameters	
• Comparative in vitro metabolism (CIVM) across species using primary hepatocytes (in vitro) • Intrinsic hepatic clearance across species using primary hepatocytes (in vitro) • Protein binding/fraction unbound in plasma across species at different concentrations (in vitro) • Binding to red blood cells (in vitro) • (Oral absorption) Intestinal permeability (in vitro - in Caco-2)	The assessment of CIVM across species is a Data Requirement listed in Reg. 283/2013. Although no OECD TG is currently available, a Scientific Opinion of the PPR Panel on testing and interpretation of CIVM studies (EFSA PPR Panel, 2021) allows the design of in vitro studies to capture qualitative and quantitative differences in metabolism profiles across species (i.e. rat, mouse, dog, rabbit, human). Human risks can be better estimated when internal concentrations in animals are understood and can be directly compared to human internal exposure using physiologically based kinetic (PBK) modelling. Information on intrinsic hepatic clearance across species, protein binding/fraction unbound in plasma across species at different concentrations (in vitro), binding to red blood cells (in vitro) and intestinal permeability (in vitro) are currently not included as data requirements in Reg. 283/2013. However, - in vitro standardised protocols to assess intrinsic hepatic clearance and plasma protein binding are available from US EPA and are included as Annex D.1 and D.2 of the current Scientific Opinion. - In vitro standardised protocols to assess intestinal permeability are already applied in the biopharmaceutical framework (rf. ICH M9[13]) and further considerations in this regard are included in Annex E.

[12] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52013XC0403%2802%29.

[13] https://www.ema.europa.eu/en/ich-m9-biopharmaceutics-classification-system-based-biowaivers-scientific-guideline.

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TABLE 9 (Continued)

Type of data	Description
In vivo toxicokinetic (TK) data from rat[a] (single and repeated oral exposure at different doses)	
• C_{max}, T_{max}, AUC, $T_{1/2}$ • Volume of distribution • Plasma clearance (time-dependent plasma concentrations) • Biliary and urinary excretion • TK data at toxicologically relevant doses	This information is already included as part of the Data Requirements stipulated in Reg. 283/2013, paragraph 5.1 stating that toxicokinetic information *'shall be generated in short and long-term studies on relevant species to enhance the value of the toxicological data generated in terms of understanding the toxicity studies'* and 5.3 *'Toxicokinetic data [..] shall be included in short term studies'*. Internationally agreed methodologies are available to generate those data (rf. Document 52013XC0403(02)), namely: - Toxicokinetics (OECD TG 417; OECD, 2010) Performing in vivo TK studies at toxicological relevant doses allows the demonstration that high systemic exposure is reached in rat (or mouse) and that the species is adequate to test the toxicological profile of the active substance. These data can also help in PBK model development and are considered critical for PBK model evaluation.

[a]If there is existing information (e.g. from substances of the same chemical classes) that indicates that rat is not a suitable surrogate for assessing human health hazard, data from other species, such as mouse, may be employed for this strategy.

The information/data gathered is integrated as schematically depicted in the flowchart reported in Figure 2 with three possible outcomes: (1) dog toxicity study can be waived, (2) dog toxicity study can be waived with further consideration on extra UF and (3) dog toxicity study cannot be waived. The outcomes are driven by the evidence assessed in the previous steps and depend on the information available. In particular, the decision of whether to include or exclude dog toxicity testing should be made on a case-by-case basis, allowing flexibility in applying the assessment strategy according to the available evidence and expert judgement, which provides a structured, evidence-informed and scientifically justified basis for decision-making.

It is relevant to mention that, in all the different steps of the assessment strategy, the analysis of the evidence comprises a weight-of-evidence (WoE) assessment (EFSA Scientific Committee, 2017) for an overall understanding of whether there is any indication that dog is less sensitive than or equally sensitive as rats (or mice).

This analysis is based on the existing information on the NAS, e.g. chemical class, the NAS' pesticidal MoA (and toxicological MoA, if known) and new information collected as part of the NAS' approval procedure, particularly kinetic data from in vitro kinetic, in vivo ADME and TK studies in rats (or mice) that would inform the PBK model development and evaluation.

The PBK model should allow prediction of the internal plasma exposure at least in dog, rat (or mouse) and human to enable cross-species comparison.

4.2.1.2 | *Running preliminary phases 1 and 2*

Preliminary phase 1 was established to allow an early evaluation of whether the waiving of dog toxicity studies can be scientifically justified with fewer data than are required for the full decision tree. It includes an assessment of species relevance based on the results of the CIVM study and MoA-related species differences. This phase provides an early opportunity to justify waiving the dog toxicity study without proceeding with the remaining parts of the strategy if one of the two following outcomes can be met:

- If the CIVM study shows that the dog has a dissimilar[14] metabolite profile compared to human, then the dog is not considered fit-for-purpose as a testing species and the dog toxicity study may already be waived with this justification.
- If the (new) active substance belongs to a chemical class with a known MoA in which rats (or mouse) are more sensitive than dogs, as substantiated by relevant data, then dog as testing species is considered not fit-for-purpose and waiving of the dog toxicity study can also be justified at this early step of the strategy. If, on the other hand, the available information on toxicological MoA for the chemical class indicates that the dog is potentially more sensitive than the rat, then dog toxicity studies may also be waived but with further consideration of an extra uncertainty factor (UF). The magnitude of such an UF depends on the available data and context of the assessment.

Preliminary phase 2 comprises the assessment of the suitability of rat (rodent) as a model for human health hazard assessment based on the results of ADME, TK and in vivo toxicity studies. It is worth mentioning that, in any case, the use of rat should be prioritised. As several toxicological endpoints (e.g. reproductive toxicity, developmental toxicity and carcinogenicity) are primarily assessed in rats (rodent species), it is crucial to evaluate whether the rat model can adequately predict and protect human health, independent of an evaluation of the sensitivity of the dog. However, if existing information (e.g. from substances within the same chemical class) indicates that rat may not be a suitable surrogate for assessing human health hazards, data from other species, such as mouse, may be employed for this strategy. The use of an alternative species should be clearly justified. Nevertheless, the successful completion of this step (see the following conditions, below, using rat as an example) is required to substantiate the justification of waiving a dog toxicity study.

[14]The EFSA PPR Panel Scientific Opinion of the Scientific Panel on Plant Protection Products and their Residues (PPR Panel) on testing and interpretation of comparative in vitro metabolism studies. EFSA Journal, 2021;19(12):6970. https://doi.org/10.2903/j.efsa.2021.6970 provides definition of unique human metabolite (UHM) and disproportionate human metabolite (DHM) as being dissimilar.

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- If there is evidence that the rat achieves systemic exposure at toxicologically relevant doses (i.e. systemic exposure demonstrated by toxicological findings) or ADME/TK data indicate internal exposure in rat, this supports the conclusion that the rat is a suitable model for hazard assessment.
- Conversely, if there is no evidence that the rat achieves systemic exposure given the absence of systemic toxicity and ADME/TK data indicate that there is insufficient internal exposure in rat, human intestinal permeability (e.g. using the Caco-2 assay) should be assessed and compared with rat ADME/TK data in parallel.
- If the rat absorption data show concordance with human Caco-2 data (i.e. low oral absorption and low permeability), then this provides justification that rat remains a suitable model for hazard assessment and the strategy may proceed to entering the full flowchart.
- If the Caco-2 assay shows moderate to high human intestinal permeability that is not consistent with limited absorption in rats, this inconsistency suggests unresolved uncertainties regarding rat suitability for hazard assessment. In this case, the strategy does not proceed to the main flowchart and additional considerations are required to address the suitability of the rat as a model for human toxicity.

4.2.1.3 | *Flowchart*

When the results of both preliminary phases (phase 1 and 2) subsequently lead to the main flowchart, i.e. preliminary phase 1 does not exclude dog toxicity testing, and preliminary phase 2 confirms the rat (or mouse) as a suitable animal species, the assessment strategy moves to the PBK modelling. The different steps of the flowchart are outlined in the following paragraphs.

Check the acceptability of PBK model: If the measured and PBK model-predicted internal exposures (C_{max} and AUC) in rats, under both single and repeated dose conditions, do not differ by more than fivefold,[15] the PBK model predictions for the tested substance are considered acceptable and the flowchart will proceed to the next step, i.e. comparison of PBK models between rat and dog. Otherwise, in vivo TK data in dog should be generated to allow comparison of internal exposure between dog and rat.

Compare PBK models between rat and dog: If the PBK model-predicted internal exposure in dogs is not more than threefold[16] higher than that in rats, the dog toxicity study can be waived. Otherwise, the flowchart proceeds to the next step, i.e. comparison of PBK models between rat and human.

Compare PBK models between rat and human: If the PBK model-predicted internal exposure in dog is higher than in rats, but the PBK model-predicted internal exposure in rats is equal to or higher than that predicted in human, then dog toxicity study can still be waived because the rat remains a suitable animal model to capture the potential human hazard. Otherwise, further in vivo TK data in dogs need to be generated and internal exposure in dogs and rats needs to be compared.

Generate in vivo TK data in dog: If the internal exposure (C_{max} and AUC) measured from the TK study in dogs is more than 10-fold[17] higher than that in rats, then the dog toxicity study cannot be waived. Otherwise, no dog toxicity study is considered necessary (i.e., the study can be waived). The PPR Panel WG acknowledges that it might be possible to still waive the dog study considering an additional uncertainty factor. It is noted that TK data in dog can be used to update the PBK model results and confirm the predictions in dog and the process can be reiterated.

Aspects based on which a dog study can be waived, can be waived with consideration of extra uncertainty factors in the risk assessment or cannot be waived, are summarised in Box 1.

[15]This threshold was selected pragmatically, based upon the outcome of the case studies and considered representative; the PPR Panel WG considers that in the context of the current scientific opinion and proof of concept case studies, it is reflecting the low tier nature of the PBK modelling and its role within a WoE assessment. Please note that model acceptance is considered case-by-case and besides prediction of Cmax and AUC, other aspects may need to be considered.

[16]This threshold was selected pragmatically, and it is not prescriptive, considering the variation in the model output in the proof-of-concept case studies.

[17]The PPR Panel WG considers that this value is justified as it covers the experimental complexity and variability of the in vivo studies. An internal analysis revealed that in the dog studies where toxicokinetic parameters were measured, interindividual variability was often very high, frequently exceeding a 10-fold difference between individual animals. This pronounced variability was particularly evident in studies conducted via dietary administration, where variability in feeding pattern among dogs can lead to uneven exposure. However, even in studies performed via gavage, where dosing is more controlled and uniform, substantial variability between individual animals was still observed.

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BOX 1 Final decision – summary

Waiving of dog toxicity studies is scientifically justified.

Dog study can be waived

- If the dog shows a dissimilar metabolite profile compared to humans OR
- If the collected information/data shows that there is no indication of a toxicological MoA suggesting that the dog is potentially more sensitive than the rat, OR
- If, after assessing the acceptability of the PBK model, the results from the PBK model indicate that the predicted internal exposure in dogs is not more than threefold higher than in rats, OR
- If, after assessing the acceptability of the PBK model, the predicted internal exposure in dog > predicted internal exposure in rat, but the predicted internal exposure in rat ≥ predicted internal exposure in human, OR
- If in vivo TK data indicate that dog internal exposure is lower, similar to or not more than 10-fold higher than the internal exposure in rat at toxicologically relevant doses.

Dog study can be waived, but extra Uncertainty Factors (UF) should be considered in risk assessment

- If the NAS belongs to a known chemical class of compounds showing a toxicological MoA indicative of higher sensitivity of dogs compared to rats. The magnitude of such an UF depends on the available data and context of the assessment.

Dog study cannot be waived*

- If the dog has higher in vivo systemic exposure (i.e. more than 10-fold) compared to the rat at toxicologically relevant dose levels for rats.

*Alternatively, the regulatory approach could also consider the applicability of an additional UF, the magnitude of which would be determined on a case-by-case basis.

4.2.2 | Outcome of the proof-of-concept case studies

Four proof-of-concept case studies were conducted to examine whether, for a given active substance, rat data supported by mechanistic and kinetic evidence can provide a sufficiently conservative basis for human health hazard assessment such that toxicity studies in dogs as a second species are not considered necessary for the assessment, or whether dog studies would be considered to provide added value.

The four case studies were selected (a) based on the available data set in relation to the minimum information required for the assessment strategy and (b) with the intention of covering different possible outcomes/cases (see below) in the hazard assessment.

- CASES 1 and 4: Dog is reported to be more sensitive than rat due to unspecific effects, (e.g. due to effects on body weight (change) or liver weight): sulfoxaflor (Case 1) and spinosad (Case 4)
- CASE 2: Dog is reported to be the more sensitive species due to putative specific toxicity (e.g. due to involvement of organic acid transporters): triclopyr
- CASE 3: Rat is reported to be more sensitive than dog: halauxifen-methyl

The following text below summarises the outcomes of the assessment strategy, the decisions drawn and the level of confidence in those assessment outcomes (see Annex C.1. for more details on the case studies and for the methodology applied; Table 10 provides a summary of the outcome).

a. **Sulfoxaflor**. A sulfoxamine insecticide with minimal metabolism in multiple species and similar metabolite profiles across species. Dogs did not show a different in vitro metabolic profile than the other species and there is no prior knowledge on toxicological MoA. Moreover, rats achieved adequate systemic exposure, and an acceptable PBK model predicted limited species differences in internal exposure (around 1.5x). **Outcome**: dog toxicity study can be waived with high confidence in the assessment.
b. **Triclopyr**. An organic acid herbicide with dogs showing higher sensitivity than rodents for renal effects as a consequence of decreased renal clearance of the substance, e.g. saturation of active tubular secretion or increased renal tubular reabsorption. Based on this information on the MoA (TD considerations), the assessment could be completed at preliminary phase 2. **Outcome:** An extra uncertainty factor (UF) could be considered in risk assessment, and the dog study can be waived with high confidence. The magnitude of such an UF depends on the available data and context of the assessment. A full assessment of all data was not performed in the current case studies and, accordingly, no UF could be proposed.

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c. **Halauxifen-methyl**. Another organic acid herbicide with similar transporter-related concerns as triclopyr (i.e. similar assessment approach, exiting the strategy at preliminary phase 1). **Outcome**: An extra uncertainty factor (UF) could be considered in risk assessment and the dog study can be waived with high confidence. The magnitude of such an UF depends on the available data and context of the assessment. A full assessment of all data was not performed in the current case studies and, accordingly, no UF could be proposed.
d. **Spinosad**. An insecticide containing a mixture of spinosyn family molecules. Rats and dogs shared similar metabolic profiles, but both differed from that of humans and there is no prior knowledge on toxicological MoA. Rat TK data showed deviation from proportionality. The PBK model developed for rats was not considered acceptable, so a comparison of internal exposure based on the PBK modelling approach was not applicable for the assessment. Following the strategy would result in the execution of a TK study in dogs, which is available for evaluation in this case. TK data revealed a higher exposure in dogs, with dose-normalised AUC values on average larger than 10-fold, thus prompting the need for further investigation. **Outcome**: dog toxicity study cannot be waived with medium confidence in the assessment, mainly due to the lack of knowledge of the exact composition of the test material in the TK studies. Alternatively, the regulatory approach could also consider the applicability of an additional UF, the magnitude of which would be determined on a case-by-case basis.
In this case of spinosad, a post hoc comparison of the 90-d studies in rat and in dog actually revealed similar NOAELs for both species, demonstrating that conducting a dog toxicity study would not have provided added value for the hazard assessment and highlighting the need for proper reporting of data.

TABLE 10 Summary of the four proof-of-concept case studies and the assessment outcomes.

	Preliminary phase 1		Preliminary phase 2	Flowchart			Outcome	
Substance	***CIVM***	***TMoA***	***Suitability of rat model***	***PBK model acceptable***	***PBK model result***	***Comparison in vivo TK rat/TK dog***	***Waiver decision***	***Confidence***
Sulfoxaflor	Similar	No indication	Y	Y	Dog/Rat 1.5x	N/A	Y	High
Triclopyr	Similar	Y	N/A	N/A	N/A	N/A	Y	High
Halauxifen-methyl	Similar	Y	N/A	N/A	N/A	N/A	Y	High
Spinosad	Similar (dog/rat), ≠ human	No indication	Y	N	N/A	> 10	N*	Medium

Abbreviations: CIVM, Comparative in vitro metabolism; N/A, not applicable; TMoA, Toxicological MoA.
*Alternatively, the regulatory approach could also consider the applicability of an additional UF, the magnitude of which would be determined on case-by-case basis.

5 | DISCUSSION

The use of two species (typically one rodent and one non-rodent, the latter often being the dog) has traditionally been the standard practice for deriving HBGVs of pesticide active substances. However, advances in toxicological science, refinement of in vitro and in silico approaches and evolving ethical considerations have called into question the necessity of maintaining the dog as a default second species. The use of dogs in regulatory testing entails specialised housing, handling and high resource demands. Moreover, under the remit of chemical legislations worldwide, the dog is used as a second species only for the approval process of pesticide active substances. This Scientific Opinion presents an opportunity to critically evaluate the relevance of dog toxicity studies in the context of regulatory assessment for pesticide active substances.

Retrospective analysis (ToR1)

The update of the retrospective analysis was built upon the work previously reported by Panzarea et al. (2022), which provides contextual insight and basis for the methodology and evaluation of the substances.

In nearly all cases where dog toxicity studies were selected for the setting of the HBGVs, the differences were attributed to experimental and biological variability, differences in study design (e.g. dose spacing or selection) or allometric scaling rather than true dog-specific sensitivity. In some other cases, the differences were attributable to unspecific effects, such as body weight changes, which are not mechanistically linked to human-relevant toxicological pathways. After accounting for study design, variability, scaling factors and specificity of toxicity, dog toxicity studies were deemed critical for setting HBGVs in only a few cases (see **Section 4.1**, Figure 1 and Annex B.2), closely matching the findings reported by Panzarea et al. (2022) and Bishop, Dellarco, and Wolf (2023). The retrospective analysis performed by Health Canada's Pest Management Regulatory Agency (PMRA) came to a similar conclusion that, for most of the pesticides, effects levels in the 1-year dog study did not substantially differ from those in other toxicity studies after considering factors like dose spacing and experimental variability (Linke et al., 2017).

The outcome from this retrospective analysis challenges the value of dog toxicity studies in most regulatory assessments and supports the development of a more targeted, evidence-based approach in determining when dog toxicity data are scientifically necessary. However, the updated retrospective analysis also revealed important gaps during the data

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evaluation, particularly the absence of standardised in vitro metabolism studies and limited availability of robust in vivo TK data sets in both dogs and rodents, which reduces the confidence in the conclusions and highlights the need for improved study design and reporting standards. The gaps provided the basis and served as criteria for developing the minimum data requirements for the tiered assessment strategy, as discussed below.

Tiered assessment strategy (ToR2)

In response to the aforementioned findings and observations, a tiered assessment strategy was developed as means to provide a structured framework to evaluate on a case-by-case basis whether the performance of dog toxicity studies is scientifically justified.

The interpretation of this assessment strategy, summarised in a decision flowchart (Figure 2), aims to support a nuanced, tiered decision-making process that balances scientific rigour with pragmatic constraints. Rather than prescribing a rigid, uniform protocol with determinate course of outcomes, the strategy encourages WoE reasoning, where available information on TK, comparative metabolism and information on systemic exposure across species using PBK modelling is integrated to allow better informed decision-making as to whether dog toxicity testing for a pesticide active substance should be included or excluded (i.e. waived). The incorporation of PBK models is particularly important, as it allows a quantitative comparison of internal exposure among rats, dogs and humans, thereby improving interspecies extrapolation (Paini et al., 2019). The rationale-based assessment strategy proposed in this Scientific Opinion is aligned with broader regulatory trends towards reducing animal testing, e.g. Bishop, Brescia, et al. (2023) and Bishop, Dellarco, and Wolf (2023); developing integrated approaches to testing and assessment (IATAs) in order to support chemical safety (OECD, 2020); European Commission's roadmap (European Commission, 2023). EFSA has increasingly advocated for the integration of new approach methodologies (NAMs) (Cattaneo et al., 2023) and the pharmaceutical sector has similarly advanced efforts to waive the second species requirements under specific conditions as reflected in the International Council for Harmonisation (ICH) S6(R1) guidance (Prior et al., 2020). However, the present Scientific Opinion extends these principles by systematically incorporating PBK modelling and human-relevant data into a tiered regulatory decision-making process for pesticide active substances.

In addition, the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R) in the UK is conducting a comparable evaluation in drug development to determine how often dogs have been critical in selecting the starting and stopping dose in early clinical studies. Although results are still preliminary (Passini et al., work in progress[18]), NC3R suggests that the use of dogs is rarely critical to predict human toxicity. This reinforces the concept applied in this Scientific Opinion that, for environmental toxicants, the regulatory toxicology is focussed on protection rather than predicting toxicity.

Case studies

The four proof-of-concept case studies presented in this Scientific Opinion collectively illustrate that a WoE approach, i.e. integrating and evaluating MoA considerations and metabolite profiles as well as comparing TK data and PBK modelling, allows to assess whether human health hazard assessment for a given pesticide active substance can be performed in the absence of dog toxicity data with medium to high confidence in the assessment. While the outcomes consistently supported that rat data suffice for the hazard assessment, the degree of certainty varied across case studies. In particular, in the case of spinosad, the confidence in the outcomes was considered medium due to high uncertainty and limited data transparency (i.e. uncertainty in levels of spinosyn A and spinosyn D in spinosad), which hampered species comparison in internal exposure (see Annex C.1. for more details).

Two key challenges were identified during this exercise. First, the minimum data requirements specified in the assessment strategy (Table 9) were generally not fulfilled for the case studies. Several dossiers lacked critical data elements, such as in vitro metabolism or robust in vivo TK data in dogs and rodents, which constrained the ability to reach definitive conclusions. This gap highlights the importance of standardised study design and data reporting. Furthermore, the inherent limitations of animal models in predicting human outcomes, especially in the context of toxicodynamics, cannot be fully overcome by kinetic modelling alone (Schmeisser et al., 2023). Second, the PBK modelling tool used for the case studies (TK Plate; version 1.0.24) was most suitable only for substances with simple kinetics, as shown in the case of sulfoxaflor.

Notwithstanding the uncertainties and limitations, the proof-of-concept case studies clearly demonstrate the practical applicability of the assessment strategy under real-world regulatory constraints. In nearly all cases, the waiving of dog toxicity studies could be scientifically justified using the available data set. In the case of spinosad, the lack of reliable data on the test item composition (a critical uncertainty with high impact on the assessment outcome) precluded the justification of waiving the dog study, resulting in the final outcome that waiving could not be supported. However, it was shown with the available 90-d toxicity studies that the NOAELs between rat and dog were similar, highlighting that the dog toxicity study did not provide added value for hazard assessment of spinosad.

[18]Review of the use of two species in regulatory toxicology studies. Available at: https://nc3rs.org.uk/our-portfolio/review-use-two-species-regulatory-toxicology-studies.

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Limitations and challenges

There are some caveats to using the proposed assessment strategy and interpreting its outcomes. It is important to note that the strategy can be best used when the minimum data requirements, especially those needed for PBK modelling, are met. The paucity of reliable data was consistently observed during both the review of the updated retrospective analysis (resulting in identification of gaps as an outcome of the review) and the case studies (leading to a number of uncertainties, especially regarding the validity of the PBK models). For the latter, having the minimum data required for the assessment strategy could have resulted in different outcomes.

The minimal PBK modelling approach applied here was also not fully capable or suitable for handling substances with complex kinetic behaviours. For such substances, more advanced PBK models with further developed functionalities together with more elaborated kinetic data, which were not available within the scope of this project, would be required to enable a more robust interpretation of the assessment. However, the main objective of this work was to demonstrate the feasibility of the proposed assessment strategy in supporting a scientifically grounded decision to waive dog toxicity testing. More broadly, the approach illustrates how integrated evidence, particularly PBK modelling, can inform regulatory decision-making on the need for dog studies. To ensure regulatory acceptance of waiving dog studies based on PBK modelling, the level of confidence in the model's predictive capacity (as established through the characterisation and validation criteria in OECD GD No. 331) must be sufficiently high to outweigh the uncertainty associated with not conducting in vivo dog studies.

Future considerations/forward strategy

This Scientific Opinion is part of a broader paradigm shift in toxicological risk assessment, moving away from the sole reliance upon in vivo animal studies towards human-relevant, non-animal methodologies. These include advanced in vitro and ex vivo systems, computational modelling and integrated testing strategies, which enhance the human relevance of toxicological assessments while reducing animal use and maintaining the same level of safety. Similar initiatives are underway in other major regulatory bodies, such as the European Chemicals Agency (ECHA, 2023), the European Medicines Agency (EMA, 2025) and the United States Food and Drug Administration (FDA, 2025), along with those already mentioned above from ICH.

Regulatory toxicology ultimately aims to protect human health and animal models do not always capture human sensitivities. Advances in molecular and cellular sciences, such as omics technologies and mechanistic pathway mapping (e.g. the adverse outcome pathway (AOP) framework), enable the integration of human-relevant animal data as well as actual human (i.e. epidemiological) data into regulatory evaluations. These approaches are already reducing the reliance on animal studies in specific areas of toxicological testing and hold promise for broader applications across other toxicological endpoints.

While this Scientific Opinion focuses on the use of dog in the toxicology evaluation of pesticide active substances, similar considerations could be applied to other higher vertebrates, such as mice and rabbits, where human-relevant mechanistic data should ultimately form the basis of hazard and risk assessment. For example, a retrospective analysis of developmental toxicity studies in rats and rabbits was conducted by Janer et al. (2008), and the authors reached a similar conclusion as this project that there were no clear indications of specifically requiring the rabbit as an additional test species for the evaluation of developmental toxicity. Lower NOAELs in animals or derived HBGVs may provide default protection, but regulatory decisions are most scientifically justified when based on mechanisms relevant to humans. The long-term objective is a regulatory framework that should rely entirely on validated human-relevant evidence, representing a fundamental paradigm shift in toxicological assessment.

6 | CONCLUSIONS

The work undertaken by the PPR Panel WG demonstrates that dog toxicity studies generally do not provide added value to the hazard assessment of pesticide active substances.

However, the available evidence indicates that a small number of cases may remain where uncertainties related to species-specific TK, mode of action or data availability do not allow a definitive conclusion on waiving the dog as a potentially informative species. Therefore, the conclusion on limited added value should be interpreted within the scope and limitations of the current data set and associated uncertainties. This evidence-based assessment approach is scientifically substantiated and could be further supported by integrating robust in vitro ADME and TK studies as well as PBK modelling into the future standard data requirement for the approval process of pesticide active substances.

Incorporating these elements would ensure that all relevant data, particularly those needed for PBK modelling, are generated in standardised manner aligned with established guidelines, thereby improving data quality, consistency and reliable reporting. Strengthening these methodological elements will not only enhance the scientific justification for waiving short-term oral toxicity testing in non-rodent species (90-day dog study) but will also improve the quality of the risk assessment by enhancing the understanding of the toxicity of active substance and its human relevance, while fostering the use of NAMs in the approval process of pesticide active substances.

Moreover, whenever the assessment strategy indicates that dog studies do not provide a meaningful additional value to the hazard assessment of pesticide active substance, these studies should not be performed for ethical reasons.

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7 | RECOMMENDATIONS

This section provides recommendations that would allow fulfilment of the conditions necessary to robustly employ the proposed assessment strategy. The recommendations are directed to the most relevant stakeholders; however, they should not be interpreted as being limited solely to the primary addressees.

The PPR Panel WG recommends the following:

European Commission:

- Update current data requirements for pesticide active substances approval process (Commission Regulation 283/2013) to meet the conditions necessary to run the proposed assessment strategy for waiving purposes (see Table 9 for further details). This would also ensure further standardisation and data quality and reliable reporting.

OECD:

- Update OECD TG 417 (OECD, 2010) to provide adequate data for running the assessment strategy and for PBK model parameterisation and evaluation. In line with previous recommendations (EFSA PPR Panel, 2025), the PPR Panel recommends updating the OECD TG 417 for in vivo toxicokinetic studies, including the measurement of toxicokinetic characteristics of the active substance other than description of the mass balance and detection of major metabolites. Besides total radioactivity, parents and major metabolites should be measured.
- Foster the development of test guidelines for in vitro ADME methods, including intrinsic hepatic clearance and plasma protein binding, to provide adequate data for running the assessment strategy and for PBK model parameterisation and evaluation. This is in line with previous recommendations (PPR Panel Scientific Opinion on QIVIVE on DNT IVB data; EFSA PPR Panel, 2025).

Applicants and Contract Research Organisations:

- Incorporate TK assessment in general toxicity studies conducted in rats to provide adequate data for running the assessment strategy and for PBK model parametrisation and evaluation. This is in line with previous recommendations (EFSA PPR Panel, 2025). Moreover, TK studies should be well conducted and properly designed, e.g. steady state reached and adequate doses selected.
- Use OECD GD No. 331 (OECD, 2021), including its reporting template, to support the development and regulatory application of PBK models.

All stakeholders:

- Ensure that PBK modelling platforms are adequate to model more complex kinetic processes, e.g. extrahepatic metabolism, enzyme capacity limitations (related to metabolic clearance, renal clearance and/or reabsorption, etc.), enterohepatic recirculation, time-dependent inhibition/induction of metabolic enzymes, tissue binding dynamics, if needed.
- Propose and extend similar initiatives to other non-EU legislations to reduce reliance on dog studies and support global convergence in regulatory practices.

In addition, possible follow-up activities were identified during the Workshop event organised by EFSA (for workshop report, see Annex F).

ABBREVIATIONS

AAOEL	acute acceptable operator exposure level
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
AKC	American Kennel Club
AOEL	acceptable operator exposure level
AOP	adverse outcome pathway
ARfD	acute reference dose
AUC	area under the curve
BMD	benchmark dose
BMDL	lower confidence limit of the benchmark dose
BMDU	upper confidence limit of the benchmark dose
BMR	benchmark response
BSA	body surface area
bw	body weight
CIVM	comparative in vitro metabolism
CLP	classification, labelling, and packaging

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CYP	cytochrome P450
DNT IVB	developmental neurotoxicity in vitro testing battery
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European union
FDA	United States Food and Drug Administration
FSH	follicle-stimulating hormone
GI	gastrointestinal
HBGV	health-based guidance value
HED	human equivalent dose
IATA	integrated approaches to testing and assessment
ICH	International Council for Harmonisation
IgA	Immunoglobulin A
ISA	individual scientific advisors
LOAEL	lowest observed adverse effect level
MoA	mode of action
MPS	microphysiological systems
MW	molecular weight
NAMs	new approach methodologies
NAS	new active substance
NC3R	National Centre for the Replacement, Refinement and Reduction of Animals in Research
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PBK modelling	physiologically based kinetic modelling
pMoA	pesticidal mode of action
PMRA	Pest Management Regulatory Agency
PPP	plant protection product
PPR Panel	Panel on Plant Protection Products and their Residues
QIVIVE	quantitative in vitro to in vivo extrapolation
QSAR	quantitative structure-activity relationship
RAC	Risk Assessment Committee
SAR	structure-activity relationship
STOTRE	single target organ toxicity after repeated exposure
TD	toxicodynamic
TG	test guideline
TK	toxicokinetic
ToR	terms of reference
UF	uncertainty factor
US EPA	United States Environmental Protection AgencyWGworking group
WG	working group
WoE	weight-of-evidence

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APPENDIX A

ADME and Toxicokinetics (TK)

This Appendix refers to the element of ADME and TK that served for the inclusion in the assessment strategy.

Over the years, there has been a definite development from sole consideration of external doses given to the animal, to measuring dose-dependent concentrations in blood, then in a selection of tissues, in target tissue(s), such as brain or liver, either in a dedicated kinetic study or in connection with toxicodynamic studies measuring various toxicity outcomes. Such data support development and refinement of PBK models and ultimately link them in integrated TK-TD models.

Need of appropriate human-relevant data and values to develop HBGVs

One of the key objectives of performing risk assessment of pesticide active substances is to protect human health from exposure to these products, and human is the eventual target species. However, human in vivo toxicokinetic studies (or any other toxicity studies as a matter of fact) using the active substance cannot be performed. Under the EU's Plant Protection Products Regulation (EC) No 1107/2009, it is explicitly stated '*For ethical reasons, the assessment of an active substance or a plant protection product should not be based on tests or studies involving the deliberate administration of the active substance or plant protection product to humans with the purpose of determining a human 'no observed effect level' of an active substance*'. Consequently, data for human health hazard assessment are or must be generated from experimental studies using animal species or various in vitro as well as in silico approaches. This intrinsically creates challenges and uncertainties regarding interspecies extrapolation.

To refine dose extrapolation, species-specific differences in absorption, distribution, metabolism and excretion (ADME) processes must be considered (Huang & Riviere, 2014):

- **Absorption**. Differences in gastrointestinal physiology, such as variation in gastrointestinal pH and transit time, can affect chemical bioavailability, requiring species-specific permeability data for accurate extrapolation (Kararli, 1995). In specific cases, potential interactions mediated by gastrointestinal transporters should be taken into consideration (Guéniche et al., 2024).
- **Distribution**. Variations in plasma protein binding and tissue composition necessitate adjustments based on species-specific partitioning data (Colclough et al., 2014). Again, transporters in various cell membranes further affect rate and extent of tissue distribution.
- **Metabolism**. Enzymatic activity, particularly involving CYP isoforms and their changes by induction or inhibition, differs significantly across species, underscoring the need for pathway-specific data (Boobis et al., 1990; Lewis et al., 1998; Martignoni et al., 2006; Turpeinen et al., 2007). Metabolic data from in vitro test systems are therefore required.
- **Excretion**. Renal clearance varies across species due to differences in glomerular filtration rate (GFR) and transporter activity, which further complicates extrapolation (Huang & Isoherranen, 2018).

Importance of estimating proper exposure characteristics

In risk assessment, it is important to demonstrate that test species are properly exposed to the substance during toxicity studies. Ideally, all major metabolites in humans should be present and identified in at least one of the tested species. Understanding of the interspecies differences in profile of the metabolites is a data requirement for pesticide active substances, although no test guideline or validated in vitro methods contribute to achieve this (see the Scientific Opinion on testing and interpretation of comparative in vitro metabolism studies (EFSA PPR Panel, 2021)).

Thus far, scaling the kinetic parameters to postulate in-life exposure across species has been used in the absence of empirical in vivo or in vitro TK evidence. Although allometric scaling has provided some insight to predict doses and toxicokinetic parameters across species, it is based on anatomical and physiological information and has several limitations. Specifically, allometric scaling is based on anatomical and physiological considerations and relations and does not account for biochemical, molecular and gene-associated factors governing xenobiotic behaviour (Boobis et al., 1990). Species-specific factors, such as enzyme activities and organ size, consequent metabolic pathways, enzyme induction and inhibition by endogenous or exogenous factors, etc., can significantly affect xenobiotic disposition, challenging the assumption of proportionality between metabolic processes and body size (Huang & Riviere, 2014). These challenges are particularly evident with compounds exhibiting non-linear toxicokinetics, extensive metabolism, active transport mechanism or interspecies differences in target expression, affinity and distribution (Sharma & McNeill, 2009). In such cases, more comprehensive and sophisticated approaches, such as physiologically based kinetic (PBK) modelling or empirical adjustments informed by metabolic data, are necessary to achieve more accurate interspecies extrapolation. Overall, what is needed is advanced techniques to replace traditional allometric scaling by incorporating in vitro data and modelling approaches that enhance prediction accuracy and address the limitations of earlier standard methods.

Macromolecular factors to understand and predict interspecies variability

Research over the last decades has clearly demonstrated that TK-participating macromolecules, binding proteins, enzymes, transporters, etc., introduce a major variable component in TK processes, which is difficult to predict by scaling or without

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actually measuring them by in vitro or in silico methods. This concerns also interspecies differences and uncertainty to predict human TK.

An often-used example concerns differences in the expression of P450 and other phase I enzymes (Martignoni et al., 2006; Turpeinen et al., 2007) as well as phase II enzymes (e.g. deficient N-acetylstransferase in dogs) among species, which result in a great variability in the spectrum and amounts of metabolites in the toxicological species compared to that observed in humans. To take this (sometimes) large variability into account in human risk assessment is sometimes challenging but should be solved to a reasonable extent by in vitro and in silico approaches. The same considerable variability concerns also transporters in cell surfaces of vasculature and tissues, binding proteins in extracellular and intracellular locations.

Methods to measure TK

In vivo TK studies represent the definitive shift from understanding dose-response relationship to internal concentration-response relationship in toxicological studies, ideally augmenting understanding chemical disposition and its relationship to toxicity outcomes in experimental species. Several parameters can be derived from in vivo TK studies.

The area under the curve (AUC) is a measure of exposure and may be obtained by integration of the exponential equation describing the curve or by non-compartmental methods using experimentally derived data points and trapezoid rule. The C_{max} is the maximum observed concentration usually achieved after an extravascular dose. Additional information obtained by TK studies are the half-life in plasma, the mean residence time (MRT), the plasma clearance [dose/AUC (mL/min/kg)], the initial volume of distribution, the volume of distribution at the steady state and the time in which C_{max} is observed (T_{max}). Also, tissue concentrations could be measured in selected tissues at available time points.

Alternative methods and advanced approaches

Advanced techniques are replacing traditional allometric scaling by incorporating in vitro data and modelling approaches that enhance prediction accuracy and address the limitations of traditional methods. For example, studies using human and animal hepatocytes or microsomes assess metabolic rates and enzyme kinetics (Rostami-Hodjegan & Tucker, 2007), facilitating in vitro to in vivo extrapolation to predict in vivo (systemic) clearance (Breen et al., 2021). PBK modelling is another powerful approach, building species-specific models that incorporate ADME parameters to simulate concentration-time profiles in humans based on animal data (Jones & Rowland-Yeo, 2013; Wedagedera et al., 2022). Monte Carlo simulations further refine these predictions by applying statistical methods to account for variability and uncertainty in ADME parameters across species (Wambaugh et al., 2019). These approaches and models are particularly more suitable for compounds with non-linear kinetics, enabling more precise dose predictions than earlier standard allometric methods.

Most of the parameters measured from in vivo TK studies can be calculated through an appropriate ADME parametrised PBK modelling. For estimating the exposure of experimental species, a minimal number of ADME parameters is necessary as part of the data entry into the model. These data would include the physicochemical properties of the chemical, the in vitro hepatic clearance in multiple species and the fraction plasma protein binding in multiple species. Also, metabolite formations and the time-dependent metabolic profiles should be of interest but usually not included in the current models except concerning quantitatively major metabolite(s). In vivo kinetic study might be necessary to test and evaluate the PBK model and should be conducted with the inclusion of doses expected to be used in toxicity studies. At high doses, saturation of absorption, protein binding, metabolism and transport system are commonly seen, and this should be considered as relevance of high dose selection.

In silico methods for predicting ADME properties include the relationship between chemical structure and biological characteristic (i.e. ADME properties). This can be assessed by a qualitative structure-activity relationship (SAR) or a quantitative SAR (QSAR). A simple and commonly used example of SAR is the Lipinski Rule of 5, which states that a chemical is more likely to be poorly absorbed when two or more of the following conditions apply: (1) number of hydrogen bond donors > 5, (2) number of hydrogen bond acceptors > 10, (3) calculated octanol-water partition coefficient (Clog P) > 5 and (4) molecular weight > 500. Therefore, critical components for a SAR study are the biological data, the chemical descriptors and the statistical method used. This area has been steadily advancing, and also major regulatory agencies and their research institutions have been actively working in this field of research.

In vitro techniques for studying metabolic activity are generally conducted in models reflecting the metabolic transformations occurring in the liver. For example, aspects of human metabolism and disposition of substances can be assessed in vitro using human liver cells obtained from organ donation or surgical resection. Hepatocyte preparations have been widely used; they contain all necessary enzymes and co-factors for both Phase I and II enzyme reactions of chemicals. Hepatocytes are well characterised and have been used to study the metabolism of endogenous molecules, toxicological mechanisms as well as xenobiotic biotransformation. In comparison to subcellular fraction such as microsomes, isolated hepatocytes represent a more sophisticated model of hepatic metabolism capable of investigating a variety of aspects of chemicals metabolism under physiological conditions.

Various hepatocyte systems differ basically for how long they are stable and viable for studies.

Isolated suspended hepatocytes are only viable for a few hours. For the investigation of chemicals with medium to high metabolic clearance, they are considered adequate test systems. The longevity of these preparations is too short to investigate time-dependent metabolism of slowly metabolised chemicals and to assess chemical-induced enzyme induction.

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However, cryopreserved hepatocytes now allow the routine storage of hepatocytes with only some loss of activity, and they could be useful for, e.g. studying interindividual variability of metabolism.

Plated (i.e. cultured) hepatocytes are a system with which long-term investigations can be performed. Primary cultures of hepatocytes are likely representing the best method to study chemicals metabolism. The major downfall of long-term culture is the substantial decrease in levels of xenobiotic-metabolising enzymes, including CYPs, within the initial 24 h of culture. Permanent hepatocyte cell lines, e.g. HepaRG™ cells have been used for some time for research and regulatory purposes and some aspects of metabolism such as induction responses have been validated (Bernasconi et al., 2019). More complex approaches such as spheroid cultures, sandwich cultures, co-cultures and liver beads, as well as liver microphysiological system (organ on a chip) have also been investigated as systems for examining chemicals metabolism and many of these methodologies have been commercialised and are available from selected companies. Especially, various liver microphysiological systems ('organ on a chip'), especially using stable liver cell lines, allow for detailed study of the various processes involved in hepatic clearance (e.g. uptake, metabolism, biliary secretion). Of course, many of these more complex set-ups have been developed for the use in toxicodynamic assessments. Older methods to study ADME and especially metabolism include the isolation of subcellular organelles, especially microsomes obtained via differential centrifugation. They have been used to study different aspects of metabolism in various tissues for decades. Also, liver slices (or tissue slices) have been used for a long time. However, preparation of liver slices requires suitable fresh tissue, so the use is dependent on tissue supply (i.e. use of animals). Thus, the system is not animal sparing although it is the closest in situ model to the in vivo situation. All hepatic architecture is maintained and flow into and out of the liver can be closely controlled. The model can provide mechanistic information and relatively clean samples containing metabolites for structural elucidation.

The integration of allometric scaling with PBK modelling, BMD methods and in vitro data provides a robust framework for deriving HEDs. This comprehensive methodology enhances accuracy by incorporating species-specific toxicokinetic data, addressing interspecies variability and enabling more accurate human dose determinations. By refining these methodological approaches, dose extrapolation is evolving into a more precise and reliable tool for assessing risks associated with diverse chemicals and exposure scenarios. This ongoing progress is critical for advancing informed regulatory standards and improving public safety.

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APPENDIX B

Toxicodynamics and modes of action (MoA)

Unspecific vs. specific toxic outcomes

Effects observed in toxicity studies can be of unspecific (related to general physiological responses) or specific origin (mechanism-driven 'narrower' responses).

Unspecific effects are general responses to a chemical and not directly related to a specific molecular or biological target; they are often results from cellular damage, or disruption of cellular or physiological functions and can occur across various tissues or organ systems. Some examples of unspecific effects are oxidative stress causing cellular injury, disruption of ATP production with consequent energy depletion, inflammation, lethargy or weight loss. An obvious question is whether and to what extent these unspecific effects can be species specific. A general assumption is that at least most of these general effects occur after substantial exposure and thus probably are at a higher end of dose-concentration curves. Regarding this Scientific Opinion, an important question is whether rat and dog differ 'substantially' in general toxicity responses. Differences are expected to be smaller than for specific adversities, but extensive analyses are lacking.

Specific effects, on the other hand, are directly related to the interaction of the substance with a particular biological target (e.g. enzymes, receptors, ion channels) and occur due to a defined mechanism of action at molecular level and at a specific cellular environment. Specific effects are well characterised and are observed frequently at doses lower than unspecific effects since they are based on specific molecular interactions. Some examples are inhibition of enzymes (e.g. acetylcholinesterase inhibition leading to neurotoxicity, an effect caused by organophosphates or carbamates), binding to ion channels (e.g. binding to open voltage-gate sodium channels, causing prolonged currents, an effect produced by pyrethroids) and DNA alkylation (leading to cell damage and cell death, an effect produced by several anti-cancer drugs).

While unspecific effects are often observed across species because they result from general physiological or cellular responses to chemicals rather than to specific molecular interaction, specific effects can differ markedly among species because they depend on molecular targets, which may vary in structure, expression levels, or function across species. It is possible that at a low dose/concentration a chemical produces a specific 'narrow' effect which is then expanded at higher concentrations to encompass other organs and functions, i.e. an unspecific effect.

Dog as a second species in drug development regulatory research

In drug development research, there has been a long debate on the use of a dog as second species and its value. There are wide views and no general consensus on the matter, but in some specific cases, dog studies have provided adequate and even necessary data for assessing safety for the continuation or termination of the drug development project (Bower et al., 2022). In any case, at least in drug development, these isolated studies demonstrate that the use of dog has been a determining factor in either continuing or stopping the development. Admittedly, drugs and pesticides offer a completely different view on using chemical substances regarding humans, drugs in doses needed for having a desired effect and avoiding harms (hopefully) and pesticides in concentrations/exposures at intended uses and afterwards not having any harmful consequences to human health. In pharmacology and clinical medicine, a certain balance between a drug-specific benefit and potential harm is acceptable. In contrast, in the toxicological evaluation of pesticide active substances, human exposure levels must be set so low that no (or statistically negligible) effects are expected. To ensure this, current regulatory assessments rely on animal-based tests, including dog studies and increasingly on human tissue-based in vitro systems.

Toxicodynamics and development of in vitro models

Within regulatory toxicology, adverse effects findings, irrespective of the test species or study type, are central to evaluating their relevance for human health risk assessment.[19] Toxicodynamics encompasses a large number of adversities, which have been and are currently measured by in vivo studies in animals using a repertoire of detection methods from visual observations to an almost endless array of behavioural, clinical, biochemical and molecular tests. On top of that, differences between humans and animal species used may be considerable and make predictions difficult and uncertain. To overcome these problems, model systems under development are mostly employing available human tissues or cell derived from human tissues. Tissue grafts and isolated cells, original or stem cells, organoids, microphysiological systems, etc. are all under intensive development, also by various national and international organisations and agencies, which are actively developing these models most often in collaboration with private industry and academic institutions. One of the implicit or explicitly stated goals – in addition to pure scientific and regulatory reasons – has been based on 3Rs and this naturally would involve in vivo dog studies. Because there are numerous adversities and underlying mechanisms, probably displaying considerable interspecies differences, the development is mostly directed towards human risk assessment by employing human cells and human cell-based test systems.

There are a large number of NAMs that have been developed or are under development especially within the pharmaceutical sector. From the food safety point of view, a recent article of Wood et al. (2025) provides a thorough and detailed

[19]Target species could also be other animals (e.g. in veterinary medicine) or environmental species (after environmental exposure of pesticides).

review on the current situation and future development of the NAMs field. One promising example of new NAMs is a set of microphysiological systems (MPS), which aim to mimic toxicokinetic and toxicodynamic processes in tissue- or organ-like setups resembling the in vivo situation. The most complete MPS are based on physiologically relevant microfluidic systems for mimicking most salient aspects of compound toxicokinetics and a repertoire of cells, aggregates and/or built-in tissues to mimic the interaction of a compound and its potential targets and ensuing effects in a more in vivo-like environment. Obviously, live cells and cell aggregates in multi-organ MPS platforms are the most difficult part to control regarding physiological and toxicological aspects for consistency and in vivo similarity. Currently, several companies and groups are developing MPS platforms for hazard assessment.

In silico approaches for safety purposes are another field that has been and is amply used in the pharmaceutical development field. For example, drug discovery and development have already been advanced with artificial intelligence and machine learning. However, the incorporation of these advanced in silico approaches into regulatory decision-making faces many challenges such as data quality, predictability, reliability and computational resource requirements. For the purposes of this Scientific Opinion, waiving dog studies, usefulness and practicability of in silico approaches may not be primary considerations.

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ANNEXES

Annex A: Working Protocol_Waiving dog studies.
Annex B.1: Reference values_Pesticides AS.xlsx.
Annex B.2: ADI and AOEL evaluation.xlsx.
Annex B.3: STOT RE classification and specific dog toxicity.xlsx.
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Annex D.1: SOP – Preparation of Hepatocyte Metabolic Stability Assay Samples.
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Annex E: Caco-2 intestinal permeability assay.
Annex F: Workshop event Report.
Annex G: Public consultation on the draft Scientific Opinion.

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