



# Initiatives to Decrease Redundancy in Animal Testing of Pesticides

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## Summary

*Two well-documented examples of studies that can be eliminated from the data requirement list without apparent impact on the quality of risk assessment are presented. Database evaluations demonstrated no clear difference in sensitivity between 3-month and 12-month dog studies. From a regulatory perspective, only two compounds were identified in which the NOAEL for the 12-month dog study was more than 2-fold lower than in any other study. Evaluation of the contribution of the mouse cancer study demonstrated that in 5% of all cases the mouse cancer study was used for reference dose setting and in 1.5% it was used for selective cancer classification. It is suggested that regulatory agencies periodically review their study requirements for redundant studies.*

*Keywords: 12 month dog, carcinogenicity mice, obsolete studies*

## 1 Introduction

In 2003 members of ILSI – HESI started a project called ACSA (agrochemical safety assessment). The purpose of this project was to evaluate how toxicity testing for agrochemicals could be done if the testing requirements were to be designed from scratch. The background of this question was the notion that our society continues to add testing requirements to an already substantial package without really looking at the overall picture, i.e. what value does each study have relative to the risk assessment and risk management process. The ACSA group was made up of distinguished academics, people from regulatory authorities and toxicologists from industry.

The results of this work were published in 2006 in a special issue of *Critical Reviews in Toxicology*. The main conclusions can be summarised as follows:

- 1) With the current study requirements we are focusing too much on long-term, very low exposure issues (which require substantial resources in toxicity testing) rather than on short-term or intermittent exposure scenarios.
- 2) Improved and early recognition of relevant toxicological effects will help to address the particular profile that a chemical may have in a more focused way.
- 3) Several studies are either of limited or no use (for risk assessment) or can be redesigned in such a way that the same amount of information can be obtained with far fewer resources.

An example of a redesigned study is the extended 1-generation study as an alternative to a full 2-generation study. The feasibility and sensitivity of such a study design has been positively evaluated for one compound by BASF and is under evaluation for several other compounds by other companies. With this study animal resources can be significantly reduced. However, an even more effective alternative to redesigning a study is to evaluate whether a study is useful at all, relative to risk assessment requirements. This is the true alternative to alternatives. Before a data requirement can be abandoned, however, careful examination needs to be done to assess the significance of the study for risk assessment purposes.

## 2 Results

There are at least two well-documented examples of studies that can be eliminated from the data requirement list without apparent impact on the quality of risk assessment. One of these studies is the 12-month dog study. The question whether dog studies are necessary at all for risk assessment has been evaluated by several authors and their conclusion was that the dog as a non-rodent species is important in risk assessment. For about 30-50% of all investigated agrochemicals (depending on the data base), the dog was the species which provided a lower no observed adverse effect level in 90 day studies relative to

the rat. Thus, the 90 day dog study provides essential information and needs to be performed.

In the past three decades it has also been necessary to perform a 12-month toxicity study in dogs for global regulatory acceptance. The large number of studies performed during this period has provided an extensive database that has been used to address the value of studies of different duration. Spielmann and Gerbracht (2001) found no clear difference in sensitivity between 3-month and 12-month studies. The distribution of the ratios between the lowest observed effect levels (LOEL) of the subchronic and chronic studies (insecticides, herbicides, fungicides) did also not show a different distribution pattern.

Doe et al. (2006) evaluated sensitivity based on NOAEL while looking at the impact on the regulatory outcome, if the 12-month dog study had not been performed. In this endeavour they looked at the lowest NOAEL of the standard set of 4 systemic toxicity studies (90-day rat, 2-year rat, 90-day dog, 1-year dog) and compared the result with and without consideration of the 12-month dog study. By the ratio of these values they found that for only two compounds the lowest NOAEL from the other three studies would be more than twice the NOAEL for the 12-month dog study. For one of these there are confounding factors with the 1-year studies. Both groups thus concluded that the 12 month dog study does not provide essential data for risk assessment.

The reason for the lack of increased sensitivity with longer duration of exposure in dogs is probably related to the total life expectancy. In rat studies the extension of exposure from 3-months to 1 year (chronic) or 2 years (cancer) takes the time of exposure relative to life expectancy from 12% to 50% or 100%. In dogs the 3-month study is about 2% of life expectancy, the 12-month study not more than 8%. In the EU the 12-month dog study is not an absolute data requirement anymore, and the US-EPA has also indicated that this study does not necessarily need to be performed. However, the world is much larger than the EU and US and we need global acceptance before a data requirement can be completely eliminated. The 12-month dog study is not necessary for risk assessment purposes. To speed up the process of acceptance of this fact, an interim solution may be suggested which should be easier to accept for those who still have doubts. In those cases in which the NOAEL/LOEL values of a 3-month rat study are lower than those obtained in the 3-month dog study – thus demonstrating the higher sensitivity of the rat in a study of similar duration – it should not be necessary to do any further dog studies.

The second study that does not contribute significantly to risk assessment is the carcinogenicity study in mice. In this study a total of 400 mice are treated for at least 18 months with a compound and are evaluated for the induction of cancer. Billington et al. (2010) evaluated a total of 195 agrochemicals with adequate cancer studies in mice for the contribution of this study type to risk assessment. With respect to the setting of a reference dose for 10 chemicals (i.e. 5% of all cases) the mouse cancer study was used. For all of these cases the NOAEL in the mouse study was close to the NOAEL obtained from other species and in 9 cases this value was between the

NOAEL and LOEL of the mouse study.

The relative insensitivity of mice was also recently demonstrated in an evaluation performed by the Fraunhofer Gesellschaft within the context of an ECETOX Task Force in which the NOAELs and LOELs for chemicals were compared. One of the results of this exercise, which involved a very large database, was that on average the NOAEL/LOEL values in rats are about 2-fold lower than in mice.

The contribution of the mouse cancer study to its actual purpose, i.e. the identification of carcinogens, is even more disappointing upon detailed examination (Billington et al., 2010). Approximately 10% of the 200 agrochemicals showed mouse-specific tumours. However, most of the tumours (70%) were liver tumours. These tumours are very often associated with a very high spontaneous background incidence of certain mice strains (e.g. the B6C3F1 strain) and are not considered to be relevant for humans (if the chemical has no genotoxic properties).

Other tumour types not necessarily relevant for human risk assessment are those related to an irritating mechanism. In the end, only in 1.5% of the investigated cases the mouse carcinogenicity study resulted in a classification as “limited evidence of a carcinogenic effect”.

These examples show that it is worthwhile to look at the usefulness of existing data requirements. It is not unlikely that we may find other studies that also serve very little purpose. A possible candidate for the list of redundant studies is the acute dermal LD<sub>50</sub>. This study has received very little attention, and there are no extended review papers available concerning its use in risk assessment. A small analysis performed using the former ECB “classlab” database indicated that a total of 4133 chemicals are classified. A total of 66 substances were classified as “harmful” following dermal administration. Out of these 16 were not classified based on oral or inhalation toxicity, but 3 of these were corrosive, leaving 13. A total of 42 substances were classified as “toxic” following dermal administration. Out of these 17 had a lower oral or inhalation classification, but 7 were corrosive, leaving 10. Three substances were classified as “very toxic” based on the dermal LD<sub>50</sub> study. Thus, the total number of selective (relevant) classifications based on LD<sub>50</sub> dermal studies is = 13+10+3 = 26. In other words, less than 1% of the relevant classifications are based on the dermal LD<sub>50</sub> study.

### 3 Conclusion

A general continuation of the three study types presented here in a “check the box” fashion is not sensible, because the data obtained with these studies are not used in risk assessment. In special cases, in which, for example, kinetic information would indicate that the dog is more appropriate than the rat, these studies could still be performed (guidelines are available), but we should all think carefully before we perform studies that serve little purpose. This can be achieved by a regular review of the usefulness of all studies requested in standard packages,



be it for agrochemical registration or for studies performed under REACH which follow a similar standard package profile. To achieve this objective, dialogue between authorities (those demanding data) and registrants (those responsible for the development of data) is essential. It is time we start this dialogue.

## References

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