Evaluation Manual for the Authorisation of Plant protection products and Biocides

NL part

Plant protection products

Chapter 4 Human toxicology; mammalian toxicity dossier

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Board
for the authorisation
of Plant protection products and Biocides

Chapter 4 Human toxicology; mammalian toxicity dossier

Category: Plant protection products

GENERAL INTRODUCTION

This chapter describes the data requirements for estimation of the human toxicological effects of a plant protection product and the active substance, and how reference values are derived within the NL framework (§2 - §2.5).

2. NL FRAMEWORK

The NL framework (§2 - §2.5) describes the authorisation evaluation for Plant protection products based on existing substances, included or not yet included in Annex I, and new active substances. A new substance is a substance not authorised in any of the Member States of the EU on 25th of July 1993.
The plant protection product that contains such substances may be authorised if the criteria laid down in the Wgb (Plant protection products and Biocides Act) 2007 [1] are met.
The product is assessed against the Rgb (Plant protection products and Biocides Regulations) [2]. The evaluation dossiers must meet the requirements in Annex II and III to Directive 91/414/EEC.

A member state may deviate from the EU evaluation on the basis of agricultural, phytosanitary and ecological, including climatological, conditions.

The NL framework describes the data requirements (§2.2), evaluation methodologies (§2.3), criteria and trigger values (§2.4) for which specific rules apply in the national approval framework or where the national framework has been elaborated in more detail than the EU framework.

Furthermore, the NL procedure described in §2 - §2.5 of this chapter can be used for evaluation of a substance for inclusion in Annex I in case no EU procedure has been described.

2.1. Introduction

For the aspect Human toxicology, mammalian toxicity dossier, the data requirements for the active substance and product do not differ from the EU framework. The NL procedure is only described if no EU procedure has been described. The evaluation methodology in the Rgb differs for some points from the EU framework (see §2.3).

2.2. Data requirements

The data requirements for chemical active substances and Plant protection products are in accordance with the provisions in EU framework, see EU part of the Evaluation Manual (§1.2).

Experiments performed after 25th of July 1993 must have been performed in accordance with GLP.

The identity of the tested substance and the tested product, and the purity of the tested substance should be clearly stated for each study.

Generally, open literature does not meet EU/OECD guidelines and is therefore usually considered as supplementary information.

The studies must be performed in compliance with the applicable guidelines. An overview of the data requirements and guidelines, and whether or not these are required for particular fields of use is given in Appendix A to Chapter 4.

Reduction of laboratory animal use and suffering currently receives much attention.
The Board prefers newly developed studies that are in line with such a regime, such as *in vitro* dermal absorption tests and *in vitro* eye irritation test. As long as these have, however, not yet been included in the applicable OECD and/or
EU Directives, a toxicologically justified statement is required if such tests are submitted.

2.2.1. Data requirements for the active substance

No difference with data requirements in EU framework. See EU framework in the EU part of the Evaluation Manual (§1.2).

Supplementary studies on the active substance
(NL: A5.08.2) (EU: IIA5.8.2)

Supplementary studies on choline-esterase inhibition
Where active substances belong to the group of organophosphates or carbamates, or where the active substance shows acetylcholine-esterase inhibition, acetylcholine-esterase activity should be monitored regularly, in particular in erythrocytes and brain.
The effect of acetylcholine-esterase inhibition should be assessed in acute studies.
The (critical) effect should then, besides all clinical symptoms also be assessed in the semi-chronic, chronic, reproduction and teratogenicity studies.
Background information on acetylcholine-esterase inhibition is given in the RIVM report with fact sheets about acetylcholine-esterase inhibition [3] and the JMPR report [4].

Supplementary studies on eye defects (cataract)
Studies on cataractogenic properties is mandatory for nitro compounds.
This study is described in several guidelines; semi-chronic research in accordance with OECD guideline 408, 409, 411 and 413 and in all chronic studies.

Supplementary studies on blood defects
Formation of Heinz bodies, methemoglobin (MetHB) or sulphhemoglobin in the blood should be determined for oxidising compounds such as nitro compounds and chloranilines.
Methemoglobin formation is considered an acute effect. Clinical symptoms that may indicate methemoglobin formation are blue colouring of the extremities and the nose.
Timing of the methemoglobin measurements is very important (not too late, or only at the end of the study).
These effects are preferably assessed in the semi-chronic, chronic, reproduction, and teratogenicity studies. Background information about methemoglobin formation (MetHB) is given in the RIVM report with fact sheets about methemoglobin [3].

Summary of mammalian toxicity and overall evaluation
(NL: A5.10a) (EU IIA5.10)
A summary is not mandatory for the NL authorisation. Where available, the applicant is requested to submit such a summary.

2.2.2. Data requirements for the product

There is no difference with the data requirements in the EU framework. See EU framework in the EU part of the Evaluation Manual (§1.2). For dermal absorption and skin sensitisation studies, further clarification of the text given in the EU framework is given below.

Dermal absorption
(see also §1.2.2 and §1.3.5 in the EU part of the Evaluation Manual)
No data need to be submitted if no risk without PPE is estimated at 100% dermal absorption. This also applies if calculations can be based on a lower default value of 10% based on physico-chemical properties. Another assumption that can be done is that dermal absorption will not be higher than oral absorption. If the AOEL is exceeded without PPE when a default value has been used for dermal absorption, dermal absorption data should be submitted.

An OECD guideline has been laid down for both types of studies. In practice, submission of one of these two studies can be sufficient, depending on the results. *In vitro* studies have been found to be very suitable to study species differences in dermal absorption. This is important because the permeability of rat skin to substances is usually higher than that of human skin.

This further implies that reliance on an *in vivo* study with the rat alone might result in an overestimation of the risk for the operator/worker.

According to the Board unnecessary use of laboratory animals must be avoided.
The Board therefore prefers that an *in vitro* study is performed. The Board only considers performance of an *in vivo* study justified if the AOEL is still expected to be exceeded on the basis of the *in vitro* study.

If data on individual tape strips are available, the first two strips will not be included in the total deliverable dose. This strategy is in line with the EFSA Agreements made in the expert meetings.

Skin sensitisation
(see also §1.2.2 in the EU part of the Evaluation Manual (§1.2).)
The Ctgb prefers, in accordance with EU requirements, a local lymph node assay (LLNA test) according to OECD guideline 429 or a Guinea Pig Maximisation Test. If a (modified) Buehler test is performed, a scientific justification must be submitted to explain why this study is preferred over the other tests. For studies with the formulated product, however, a (modified) Buehler test is not simply rejected. The results of the sensitisation study with the substance and the fact whether the formulation contains co-formulants with components with sensitising properties are always taken into account.
In case a (modified) Buehler test has been submitted, a scientific justification should always be provided why this test has been performed.
For clarification, a number of situations are described below:

- Where the maximisation study with the substance is negative and the formulation contains no co-formulants with sensitising properties, the Ctgb will accept a well performed (modified) Buehler test.
- Where the maximisation study with the substance is negative but the formulation contains co-formulants with sensitising properties, the Ctgb will use mathematical methods (see 99/45/EC) to decide on labelling. Possible negative results from a (modified) Buehler test with the formulation are not simply accepted. The results of an LLNA or maximisation study with the formulation, if available, overrule a possible calculation.

- Where the maximisation study with the substance is positive, the Ctgb will use the calculation rules to decide on labelling (see 99/45/EC). Possible negative results from a (modified) Buehler test with the formulation are not simply accepted.
  The results of an LLNA or maximisation study with the formulation, if available, overrule possible a calculation, and the results of the (modified) Buehler.
- Where a (modified) Buehler test with the formulation is clearly positive, such a study is in principle acceptable and performance of an LLNA or maximisation study is not required.

If, according to the applicant information with regard to acute, oral, dermal and inhalatory toxicity, and skin and eye irritation and sensitisation of the formulation obtained by calculation is sufficient, the applicant should submit a toxicologically-based justification as indicated in 99/45/EC.

2.3. Derivation of endpoints and reference values

The evaluation methodologies of toxicity studies for chemical active substances and Plant protection products are in accordance with the provisions described in EU part of the Evaluation Manual (§1.3). Further clarification of the EU procedures and specific rules applying to the national approval framework are given below. In the Netherlands a tiered approach is used in the operator, worker and bystander risk assessment, see Chapter 4 Human toxicology, risk operator, worker and bystander of the NL part of the Evaluation Manual, §2.4.1.

2.3.1. Derivation of the list of endpoints for human toxicology

This section gives a further description of the information given under data requirements (see §1.2 in the EU part of the Evaluation Manual and 2.2).

Where for a certain aspect (e.g., mutagenicity, reproduction toxicity etc.) no qualitative and/or insufficient quantitative research is available, no final conclusion or endpoint can be derived for this aspect. Additional information is necessary in such cases.

Each study is summarised separately in the toxicological summary and, where possible, the 'No Observed Adverse Effect Level' (NOAEL) is derived.
The following factors are, among others, taken into account in the derivation of, e.g., a NOAEL [5]:

- toxicological relevance of the effect (adverse versus non-adverse);
- toxicological relevance of the effect for man;
- dose-response relationship;
- statistical significance of the effect;
- relationship between the effect and other effects that occur at higher dose levels.

International developments, as published by WHO, JMPR and OECD, are also taken into account when determining whether certain effects are relevant.
The dose is expressed in mg/kg bw/day. Where food intake is not reported in a study, standard conversion factors are used to convert from ppm to mg/kg bw/day. For mouse, rabbit, rat, and dog the dose in ppm is divided by 10, 33, 20 and 40, respectively, in case of young adult laboratory animals [6,7]. A conversion factor of 15 is used for conversion from ppm to mg/kg bw/day for a reproduction toxicity study with the rat.

Genotoxicity and carcinogenicity

The standard genotoxicity package normally includes three *in vitro* tests and one *in vivo* study. When a substance is negative in the three *in vitro* tests and in an *in vivo* test, it is generally assumed that the substance is not genotoxic.

Where one or more of the *in vitro* tests show a positive result, the substance is intrinsically genotoxic. A specific *in vivo* genotoxicity test is in that case required (see data requirements) with, generally, rat and mouse as animal species.
Where the *in vivo* test is positive as well, the substance is considered genotoxic. Subsequently the relevance of this finding for man is assessed. This may require supplementary research into the mode of action of the substance.

For substances holding a risk of tumour formation through direct effects on genetic material (genotoxic carcinogenesis) a risk value should be determined. This is indicated in the Rgb, see text below (in Dutch):

Artikel 2.7. Gezondheidskundige norm

4. In aanvulling op het tweede lid bepaalt het college in geval van blootstelling aan stoffen met kankerverwekkende effecten zonder toxicologische drempelwaarde het risicogetal. Dit risicogetal wordt overeenkomstig het eerste lid aangemerkt als gezondheidskundige norm.

Other mechanisms of tumour formation allow a threshold approach: there is an exposure level at which the effect does not occur.

For substances with intrinsic (*in vitro*) genotoxic properties, but for which these properties are not expressed in *in vivo* genotoxicity tests and in chronic/carcinogenicity studies, the approach is as follows:
Where sufficient (animal) experimental evidence exists for the non expression of genotoxicity *in vivo,* a limit value approach is followed in the risk assessment (the NOAEL from the chronic/carcinogenicity studies, usually based on general toxic effects because tumour formation is usually not the most sensitive effect). However, the margin between the NOAEL for tumour formation and the overall NOAEL of the study should be taken into account. No additional safety factor is required if this margin is large enough (about a factor of 10).

2.3.2. Derivation of the ADI

For derivation of the ADI, the Netherlands applies the same method as in the EU (see §1.3.2 in the EU part of the Evaluation Manual).

2.3.3. Derivation of the health based reference value

The text from the Rgb specifically referring to the derivation of a health based reference value for the operators is given below (in Dutch). The Rgb indicates that a health based reference value for systemic effects should be derived, based on either the AOEL or the limit value from the ‘Arbeidsomstandighedenbesluit’, the Tolerable Limit Value (TLV), and formerly known as MAC-value.

Artikel 2.7. Gezondheidskundige norm

1. Het college bepaalt voor alle voor de toelating relevante blootstellingen de gezondheidskundige norm voor systemische effecten op de gezondheid door blootstelling via de orale, dermale en inhalatoire blootstellingsroute.
2. De blootstelling wordt voor iedere blootstellingsroute voor de systemische effecten op de gezondheid uitgedrukt in mg/persoon per dag en voor de inhalatoire blootstellingsroute van vluchtige stoffen tevens uitgedrukt in mg/m^3 inademingslucht.

3. Het college maakt bij de bepaling van de gezondheidskundige norm gebruik van het Acceptable Operator Exposure Level (AOEL) zoals voortkomend uit de beoordeling van de werkzame stof in het gewasbeschermingsmiddel door de Commissie van de Europese Gemeenschappen, bedoeld in de artikelen 5 en 6 van richtlijn 91/414/EEG, en de grenswaarde zoals vastgesteld krachtens art. 4.3, eerste lid, van het Arbeidsomstandighedenbesluit.
4. In aanvulling op het tweede lid bepaalt het college in geval van blootstelling aan stoffen met kankerverwekkende effecten zonder toxicologische drempelwaarde het risicogetal. Dit risicogetal wordt overeenkomstig het eerste lid aangemerkt als gezondheidskundige norm.
5. Het college bepaalt voor zover mogelijk op grond van het dossier in alle gevallen de gezondheidskundige norm voor lokale effecten op de gezondheid door blootstelling voor de dermale en inhalatoire blootstellingsroute voor kortdurende alsmede langdurige blootstelling. Deze effecten worden:
– bij de dermale effecten uitgedrukt in mg/persoon per dag en
– bij inhalatoire effecten uitgedrukt in mg/m^3 in de inademingslucht per persoon per dag.

6. Wanneer uit de risicobeoordeling bedoeld in bijlage VI, deel I, onderdeel C. punt 2.4.1, bij richtlijn 91/414/ EEG, blijkt dat de risico-index zonder gebruik van persoonlijke beschermingsmiddelen groter is dan 1, wordt de gezondheidskundige norm met uitzondering van die voor kankerverwekkende effecten zonder toxicologische drempelwaarde, opnieuw berekend met behulp van de methode allometrische extrapolatie en wordt de risico-index opnieuw bepaald.
7. Wanneer na toepassing van het zesde lid de risico-index bij de dermale blootstellingsroute groter is dan 1, wordt bijlage III, deel A, punt 7.3, bij richtlijn 91/414/EEG toegepast en wordt deze risico-index met behulp van de daaruit verkregen nieuwe informatie opnieuw bepaald.
8. De aanvullende beoordeling, bedoeld in het zesde en zevende lid, aanhef, heeft voorrang op een beoordeling aan de hand van de aanvullende gegevens, genoemd in bijlage III, deel A, punten 7.2.1.2. en 7.2.3.2, bij richtlijn 91/414/EEG, tenzij de aanvrager de aanvullende gegevens al heeft verstrekt.

Artikel 2.7a. Omstander beroepshalve aanwezig [Treedt in werking per 01-01-2010]
1. Voor de bepaling van het risico voor een persoon die zich beroepshalve bevindt in de nabijheid van de gebruiker, zijn de artikelen 2.5 en 2.7 van overeenkomstige toepassing.
2. Het college schat de kwantitatieve blootstelling aan het gewasbeschermingsmiddel, bedoeld in bijlage III, deel A, punt 7.2.1.1 bij richtlijn 91/414/EEG, zonder daarbij rekening te houden met het effect van persoonlijke beschermingsmaatregelen. Het college gebruikt voor de inschatting van de blootstelling het model EUROPOEM II.

Artikel 2.7b. Risico niet-professionele gebruiker [Treedt in werking per 01-01-2010]
Bij de beoordeling van gewasbeschermingsmiddelen voor niet-professioneel gebruik maakt het college een inschatting van het risico van blootstelling voor mens en dier op basis van zijn deskundigenoordeel.

In practice, the AOEL will generally be the most critical value.

In Tier I the same method applies as in the EU (see §1.3.3 in the EU part of the Evaluation Manual) for derivation of the AOEL/AEL.

However, deviations from an established EU-AOEL are possible in case the exposure scenario of the proposed use in the Netherlands is not covered by the EU-AOEL (e.g. a semi-chronic AOEL is derived in the EU while in the Netherlands chronic exposure is possible).

In Tier II of the operator, worker and bystander risk assessment an AOEL/AEL based on the allometric extrapolation method may be used. Such an AOEL is also known as the NL-AOEL and the method was described in HTB 0.2 referring to a method developed by TNO [8]. Discussion and weighing of the total data package is an important element for the final choice of the overall assessment factor comprising various (sub-)factors related to:
Interspecies differences
Intraspecies differences
Compensation for duration of exposure
Type of critical effect
Confidence in the available toxicological dossier (database)

These assessment factors are described below.

Intraspecies differences
This factor compensates for the larger variation in sensitivity within the population of exposed workers when compared to the relatively small group of exposed experimental animals.
For the professional population a factor of 3 is applied.
When the AOEL is derived from a developmental study in which developmental effects are found, which are not the results of maternal toxicity, a factor of 10 is applied for the professional population. In that case, no distinction is made between the professional and the general population.
For substances that have already been extensively investigated in man, a smaller factor can be used, since a larger part of the variation in sensitivity has already been taken into consideration. This must be motivated in the decision-making stage.

Compensation for duration of exposure
If the risk for semi-chronic or chronic exposure is assessed on the basis of studies with a shorter duration an assessment factor is applied.

For extrapolation from sub-acute exposure (21/28 days) to semi-chronic exposure (10-20% of the lifespan of the animal species concerned), and from semi-chronic to chronic (i.e. approximately lifelong) exposure the default factor is 10. For extrapolation from sub-acute to chronic exposure a default factor of 50 is used [9].
A deviation from the default factor must be justified in the decision-making stage.

Type of critical effect
For the critical (the most sensitive) effect, the default value is 1. The nature of the critical effect can however give rise to a larger factor. For example, microscopically visible brain damage may indicate application of a factor higher than one.
A deviation from the default value must be justified in the decision-making stage.

Confidence in the available toxicological dossier (database)
For the reliability of the available dossier or database a default value of 1 is used. For incomplete dossiers, old studies that do not comply with current guidelines anymore etc. a larger factor can be considered. A deviation from the default value must be justified in the decision-making stage.

If, in the absence of a useful NOAEL, an AOEL is derived from the lowest observed adverse effect level (LOAEL), an additional factor can be applied. A factor of 10 is used as a default value. Information in the dossier, particularly concerning the slope of the dose-respons curve, the distance to the probable NOAEL etc. can lead to the use of another factor. The choice must be motivated in the decision-making stage.

If there are significant limitations in the available toxicity data, supplementary data should be generated, as no factor can compensate for these limitations.

There are a number of supplementary remarks:

In the Netherlands, the AOEL/AEL is usually also expressed in mg/person/working day. An average body weight of 70 kg is assumed as default value for professional operators (including workers) and 63 kg for the non-professional operator and worker.

Certain applications in the Netherlands are also performed by contract workers. A TNO report with the results of a survey among contract workers was published in 2001[10]. This survey was performed by the sector organisation of contract workers in the Netherlands, Cumela. Cumela conducted a new survey in 2004. The results show that contract workers may operate in the following crops (with a proportion contract labour >10%): maize, cereals, beet, potatoes, onions, grassland, asparagus, vegetables for processing, and other field vegetables, other vegetable crops (e.g., oilseed rape, flax, oil-containing crops), tree nursery stock, public parks and gardens, recreation grasses and uncultivated land.
The expected exposure duration for a contract worker will be evaluated per application, on the basis of which a decision will be taken whether a semi-chronic or chronic AOEL needs to be derived.

2.3.4. Derivation of the ARfD

The NL method for derivation of the ARfD is the same as in the EU (see §1.3.4 in the EU part of the Evaluation Manual).

2.3.5. Derivation of the dermal absorption value for the list of endpoints

The Netherlands follows the EU Guidance document on dermal absorption [11] (see also §1.3.5 in the EU part of the Evaluation Manual), in accordance with the procedure in the EU, for derivation of the human dermal absorption value for the list of endpoints.

The dermal absorption given in the EU list of endpoints does not necessarily need to be used for the calculation of the systemic exposure for NL applications. The extent of dermal absorption is affected by various factors such as co-formulants and exposure level (area dose) and is not an intrinsic property of the substance.

2.3.6. Combination of two or more active substances in a product

Combination toxicity should be determined for a plant protection product that contains several active substances, as well as for combinations of Plant protection products of which the combination (tank mix) is recommended in the instructions for use.
The acute toxicity of the product is known (= data requirements product) for Plant protection products with several active substances. A risk assessment should, however, also be performed for repeated exposure to the combination of two or more active substances.

This is not part of the data requirements. This aspect is, however, given attention in the national evaluations. Combined exposure to substances may possibly lead to a different toxicological profile than the profile derived for the individual substances because they may interact.
Aspects considered in the evaluation include the toxicological profile (critical effect, mode of action), metabolism of the substances, and whether the substances cause enzyme induction. Two or more substances may have an additive, synergistic or antagonistic effect on each other's activity. A synergistic or antagonistic effect, however, requires that exposure takes place at or near the level at which undesirable effects of the individual substances may be expected (in comparison with the AOEL/ADI/ARfD).

Current Annex III requirements in the EU only concern combinations of products.

2.4. Approval

The actual decision whether a plant protection product can be authorised follows from the risk evaluation for operator, worker, bystander and consumers, which is elaborated in Chapter 4 Human toxicology, risk operator, worker and bystander and Chapter 5 Residues, risk for consumers in the NL part of the Evaluation Manual.

2.5. Developments

Developments in the EU framework (see under §1.5 in the EU part of the Evaluation Manual) will also affect the data requirements and evaluation methodologies in the NL framework in view of the aim of the largest possible harmonisation of data requirements and evaluation methods.

3. REFERENCES

1 Wgb
NL acts, decisions, orders, etc. can be obtained via http://wetten.overheid.nl/
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