Azadirachtin
Evaluation of Classification and Labelling Proposal with regard to Skin Sensitisation

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I, the undersigned, hereby declare that the present report has been prepared by GAB Consulting GmbH, Hinter den Höfen 24, 21769 Lamstedt, Germany.

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17 November 2014

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1 Executive Summary

Azadirachtin is a refined medium polarity extract from the kernels of the Neem tree. It is approved in the EU as active substance for plant protection products.

RMS Germany prepared a CLH-Dossier to define an appropriate harmonized classification for Azadirachtin and, regarding skin sensitisation, proposed classification in Category 1 (H317).

Based on the submitted data and considering also the experimental evidence obtained with the plant protection products, Azadirachtin did meet the criteria laid down in the CLP regulation to be classified with Skin sensitisation Category 1B (H317 - May cause an allergic skin reaction).

2 Introduction


Azadirachtin is one of very few insecticides permitted in organic farming. It has been used safely for 20 years as an active ingredient in several plant protection products.

Regarding skin sensitisation, classification in Category 1 (H317) is proposed. The available data on skin sensitisation is discussed in the following. According to this, Category 1B is proposed.

3 CLH-Proposal – Skin sensitisation

In the CLH report (Proposal for Harmonised Classification and Labelling) the RMS Germany concluded regarding classification and labelling that

Results with NeemAzal and NPI 720 lead to a classification in category 1B, whereas results with Fortune Aza lead to category 1A. Considering the contradictory categories, it is proposed to place Azadirachtin into category 1 (without sub categories), see Part B, Point 4.6.1.4 (page 24).

4 Available Data

Data on the skin sensitisation of Azadirachtin are available for three technical extracts: NeemAzal and Fortune Aza were tested according to the protocol of Magnusson & Kligman, whereas NPI 720 was tested according to Buehler, i.e. without adjuvant. Fortune Aza, NeemAzal, and NPI 720 showed sensitising potential upon skin contact.

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Table 1: Summary of skin sensitisation (taken from CLH report, Part B, Point 4.6.1.1, Table 19)

<table>
<thead>
<tr>
<th>Animal species strain</th>
<th>Number of animals</th>
<th>Doses</th>
<th>Result</th>
<th>Reference Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig, Dunkin Hartley albino</td>
<td>20 M treated 10 control</td>
<td>Intradermal: 5 % (w/v) in acetone/alembicol Dermal: 80 % in acetone</td>
<td>Sensitising (M&amp;K) [all animals sensitised]</td>
<td>NeemAzal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOX9700507 OECD TG 406</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig, Dunkin Hartley albino</td>
<td>20 M treated 10 control</td>
<td>Intradermal: 0.5 % (w/v) in acetone/alembicol Dermal: 60 % in alembicol</td>
<td>Sensitising (M&amp;K) [all animals sensitised]</td>
<td>Fortune Aza</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOX2005-2384 OECD TG 406</td>
</tr>
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</tr>
<tr>
<td>Guinea pig, Hartley albino</td>
<td>10 M treated 10 control</td>
<td>Dermal: 25 % (w/v) in ethanol</td>
<td>Sensitising (Buehler) [2/10 animals sensitised]</td>
<td>NPI 720</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOX2005-2383 OECD TG 406</td>
</tr>
</tbody>
</table>

Table 2: CLP criteria for skin sensitisation

<table>
<thead>
<tr>
<th>Category 1A (H317):</th>
<th>Category 1B (H317):</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 30 % responding at ≤ 0.1 % intradermal induction dose or</td>
<td>≥ 30 % responding at ≤ 0.1 % intradermal induction dose or</td>
</tr>
<tr>
<td>≥ 60 % responding at &gt; 0.1 % to ≤ 1 % intradermal induction dose</td>
<td></td>
</tr>
</tbody>
</table>

Comparing the test results with the CLP criteria, the rapporteur stated in the CLH report: *Results with NeemAzal and NPI 720 lead to a classification in category 1B, whereas results with Fortune Aza lead to category 1A. Considering the contradictory categories, it is proposed to place Azadirachtin into category 1 (without sub categories).*

However, although a 100% response was observed in a Maximisation test according to Magnusson Kligman with an intra-dermal induction concentration of 0.5% Fortune Aza, classification with Category 1A is not warranted.

Indeed, experimental results from skin sensitisation tests with the plant protection products (summarised in Table 4, full report summaries taken from the DAR on Azadirachtin are given in Point 5 below) demonstrated that the products with concentrations of 3-26% Azadirachtin extract were not sensitising indicating that the Azadirachtin is not a strong skin sensitiser.

In particular, a Buehler test conducted with the product based on Fortune Aza (Allan, Coleman, 1997d) was conducted with the undiluted product, corresponding to a concentration of 26% Azadirachtin extract. Thus, meeting the CLP criteria for Category 1B of >20% topical induction dose but with no indication of skin sensitisation response in any of the 20 test animals.
Table 4  Skin Sensitisation studies with Plant Protection Products

<table>
<thead>
<tr>
<th>Number of animals (Guinea pigs)</th>
<th>Test substance Concentration of Azadirachtin</th>
<th>Doses product (Induction)</th>
<th>Doses active substance (Induction)</th>
<th>Result</th>
<th>Reference Report No Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>M&amp;K 20 M treated 10 control</td>
<td>NeemAzal T/S 3% Azadirachtin Extract NeemAzal</td>
<td>Intradermal: 5 % in sesame oil Dermal: 100% undiluted</td>
<td>Intradermal: 0.15% Dermal: 4.5%</td>
<td>Not sensitising [no animal sensitised]</td>
<td>Kramer, 1998 981042830 OECD 406</td>
</tr>
<tr>
<td>Buehler 20 M treated 10 control</td>
<td>NeemAzal Formulation 4.5% Azadirachtin Extract NeemAzal</td>
<td>Dermal: 100% undiluted</td>
<td>Dermal: 4.5%</td>
<td>Not sensitising [no animals sensitised, 2/20 inconclusive]</td>
<td>Allan, Coleman, 1997c EIP 19/951048/SS OECD 406</td>
</tr>
</tbody>
</table>

5 Report summaries of skin sensitisation tests with Azadirachtin formulations

The following study summaries of skin sensitisation studies performed with formulations containing Azadirachtin were taken from the DAR on Azadirachtin of 19 November 2007, see Volume 3, B.6, Point B.6.11.1.6 (page 299 ff). They show that these formulations had no sensitising effects.

**Reference:** TRF IIIA 7.1.6
**Deviations:** None
**GLP:** Yes (certified laboratory)
**Acceptability:** The study is considered to be acceptable.

**Material and Methods:**
Test material: NeemAzal-T/S (dark brown liquid; 1 % azadirachtin-A; lot/batch #: 100898); vehicle: Sesame oil.
Test animals: Albino Guinea Pig, Dunkin Hartley; source: Harlan Winkelmann, Borchen, Germany; age: Younger than one year; weight at dosing: 365 – 501 g.

Animal assignment and treatment:

**Pre-test:**
In order to assess test item concentrations to be applied in the main test a pretest was performed. Intradermal injections were done with a total of 3 test substance concentrations, 1%, 3% and 5% NeemAzal-T/S in sesame oil (v/v). Each of 3 animals received 2 different concentrations in duplicate (0.1 mL/site) in the clipped scapular region. Dermal reactions were assessed 24, 48 and 72 hours after treatment. Epidermal application was carried out in a concentration range from 25%, 50% and 100% in sesame oil (v/v). Two different concentrations (0.5 mL each) were applied per animal to the clipped flank (2 x 3 cm) using semi-occlusive dressings. After 24 hours, the dressing was removed, the skin cleaned with water and the dermal reactions assessed 24 and 48 hours later. A severe erythema was noted at 100%, moderate erythema at 50 and no erythema at 25%. No oedema were observed. There were no skin reactions observed.
Main test:

Intradermal induction (experimental group: 10 animals): For induction, on day 0, the scapular region was clipped and the following three pairs of intradermal injections (0.1 mL/site) were made:

- A 1:1 w/w mixture of Freund’s Complete Adjuvant with water for injection
- NeemAzal-T/S 5% in sesame oil
- NeemAzal-T/S 5% in a 1:1 (v/v) mixture Freund’s Complete Adjuvant and sesame oil.

On day 6 the scapular area between the injection sites was clipped and rubbed with 0.5 mL of 10% sodium lauryl sulfate in vaseline - this concentration causes a mild inflammatory reaction.

Epidermal induction: On day 7 the clipped area was treated with 1 mL of un diluted NeemAzal-T/S for 48 hours using a filterpaper covered with impermeable plastic tape and fixed with an elastic adhesive bandage. The control animals were treated as described for the experimental animals except that, instead of the test substance, vehicle alone was administered.

Challenge: For challenge on day 21 both flanks of all animals were clipped and treated by epidermal application of 25% NeemAzal-T/S in sesame oil (1 mL on the left flank) or sesame oil (right flank), using patch test plasters. The patches were held in place with tape and subsequently elastic bandage. The dressing was removed after 24 hours exposure and the skin cleaned of residual test substance and vehicle using water. The treated sites were assessed for challenge reactions 24 and 48 hours after removal of the dressing.

Findings:

No mortality occurred and no symptoms of systemic toxicity were observed. Body weights and body weight gain remained in the same range as controls. No skin reactions were observed in animals of the treatment group upon challenge with NeemAzal-T/S.

An earlier test with ethyl p-aminobenzoate as positive reference substance (performed in parallel during September 1998) resulted in allergic reactions and has shown the sensitivity of the guinea pig strain used.

Conclusions:

The test substance NeemAzal-T/S exhibited no dermal sensitisation potential under the test conditions used according to Magnusson and Kligman. On the basis of this study NeemAzal-T/S does not require classification/labelling as sensitising.

Reference: TRF IIIA 7.1.6
NeemAzal Formulation - Skin Sensitisation in the Guinea Pig.
Report-no. EIP 19/951048/SS. TOX9700521
Corresponds to OECD Guideline 406 (1992)
Deviations: None
GLP: Yes
Acceptability: The study is considered to be supplementary.
(Buehler test with only 3 induction applications)

Executive summary:

The skin sensitisation potential of NeemAzal-T/S was assessed using guinea pig according to Buehler with twenty test and ten control animals. The study comprised of three induction applications with undiluted NeemAzal-T/S, following a challenge with undiluted NeemAzal-T/S. A re-challenge was carried out with NeemAzal-T/S diluted with distilled water to 50 and 25%. At the first challenge, irritant responses were seen for the control animals preventing a precise evaluation of the test animals. Therefore, a second challenge was carried out with test substance at the lower concentration. Based on the results of the second challenge NeemAzal-T/S did not produce evidence of skin sensitisation in eighteen of twenty animals. The remaining two animals gave inconclusive responses.
Material and Methods:
Test Material: Fortune Aza 3% EC; Brown liquid; Purity: 2.42% Azadirachtin A+B, HPLC analysis results of Fortune AZA 3 % EC conducted at Huntingdon Life Sciences Department of Analysis were 1.84% Azadirachtin A (date of assay 6 March 1996) and 1.90% Azadirachtin B (date of assay 29 April 1996); Lot/Batch #: 220040595; Vehicle: None. Test animals: male Albino Guinea Pig, Dunkin Hartley; Source: D. Hall, Newchurch, Staffordshire, England; Age: approximately 6-7 weeks; Weight at dosing: 290-349 g.

Animal assignment and treatment:
Pre-test:
Epidermal application was carried out in a concentration range from 30 % to 100 %. Four different concentrations (30, 50, 70 and 100%; 0.5 mL each) were applied per animal to the clipped flank (2 x 3 cm) using semi-occlusive dressings. After 6 hours, the dressing was removed, the skin cleaned with water and the dermal reactions assessed 24 hours later. There were no skin reactions observed.

Main test:
Experimental group (20 female animals): For induction, on day 1, left shoulder region was clipped and the clipped area was treated with 0.5 mL of an undiluted Fortune Aza 3% EC for 6 hours using a surgical gauze covered with impermeable plastic tape and fixed with elastic adhesive bandage. The control animals (10 female) were treated as described for the experimental animals except that, instead of the test substance, dry patches were administered.

The induction was repeated on days eight and fifteen.

For challenge two weeks after the final induction one flank of each animal was clipped and treated by epidermal application of 100 % Fortune Aza 3% EC, 0.15 mL each, using patch test plasters. The patches were held in place with Blederm tape and subsequently Elastoplast elastic bandage. The dressing was removed after 6 hours exposure and the skin cleaned of residual test substance and vehicle using water. The treated sites were assessed for challenge reactions 24 hours after removal of the dressing.

Findings:
No mortality occurred and no symptoms of systemic toxicity were observed. Body weights and body weight gain remained in the same range as controls. No signs of irritation were observed upon dermal application of up to 100% Fortune Aza 3% EC. No skin reactions were observed in control animals or in treated animals.

An earlier test with formalin as positive reference substance (performed regularly, 25.2.1995) resulted in allergic reactions and has shown the sensitivity of the guinea pig strain used.

Conclusions:
The test substance Fortune Aza 3% EC/Oikos did not produce evidence of skin sensitization potential under the test conditions used (Buehler test with only 3 induction applications).
6 Overall conclusion

In summary based on the submitted data and considering also the experimental evidence obtained with the plant protection products, Azadirachtin did meet the criteria laid down in CLP regulation to be classified with Skin sensitisation Category 1B (H317 - May cause an allergic skin reaction) only.

7 References

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Title</th>
<th>Testing Facility</th>
<th>Owner / Source (where different from owner)</th>
<th>Report No</th>
<th>GLP or GEP status (where relevant)</th>
<th>Published or not</th>
<th>Owner</th>
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<tbody>
<tr>
<td>Allan, S., Coleman, D.</td>
<td>1997a</td>
<td>Neemazal technical skin sensitisation in the guinea-pig</td>
<td>Huntingdon Life Sciences Ltd., Huntingdon, UK</td>
<td>Trifolio-M GmbH</td>
<td>Report no. EIP 10/950818/SS</td>
<td>GLP: yes</td>
<td>Published: no</td>
<td>TRF</td>
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<tr>
<td>Allan, S., Coleman D.</td>
<td>1997c</td>
<td>NeemAzal Formulation skin sensitisation in the guinea-pig</td>
<td>Huntingdon Life Sciences Ltd., Huntingdon, UK</td>
<td>Trifolio-M GmbH</td>
<td>Report-no. EIP 19/951048/SS</td>
<td>GLP: yes</td>
<td>Published: no</td>
<td>TRF</td>
</tr>
<tr>
<td>Allan, S., Coleman D.</td>
<td>1997d</td>
<td>Fortune Aza 3% EC - Skin Sensitization to the Guinea-Pig</td>
<td>Huntingdon Life Sciences Ltd., Huntingdon, UK</td>
<td>Oxon Italia S.p.A.</td>
<td>Report-no. FBT 19/952235/SS</td>
<td>GLP: yes</td>
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