

<p style="text-align: center;">Azadirachtin Evaluation of Classification and Labelling Proposal with regard to Skin Sensitisation</p>
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Author:

Prof. Dr. Wolfgang Pfau
GAB Consulting GmbH
Hinter den Höfen 24
21769 Lamstedt
Germany

Phone: +49 4773 8889 141
Fax: +49 4773 8889 20
Email: wolfgang.pfau@gabconsulting.de

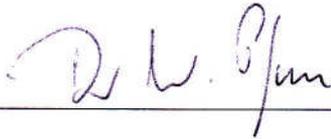
AUTHENTICATION

Report No.: 234379-A2-050206-01
Report Title: Azadirachtin: Evaluation of Classification and Labelling Proposal with regard to Skin Sensitisation

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

Signature

Date



17 November 2014

Prof. Dr. Wolfgang Pfau
certified Toxicologist (DGPT, EUROTOX)

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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 a refined medium polarity extract from the kernels of the Neem tree. Azadirachtin was included into Annex I of Directive 91/414/EEC by Commission Implementing Directive 2011/44/EU (13 April 2011) for use as insecticidal pesticide in the EU. Following entry into force of Regulation (EC) No 1107/2009, Azadirachtin is now included in the Annex to Commission Implementing Regulation (EU) No 540/2011¹.

Azadirachtin is one of very few insecticides permitted in organic farming². 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 NeemAzaal 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: NeemAzaal 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, NeemAzaal, and NPI 720 showed sensitising potential upon skin contact.

¹ (OJ L 153/77, 01 January 2009 and OJ L 153/18, 01 June 2004, p. 1-186).

² Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control

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

Animal species strain	Number of animals	Doses	Result	Reference Method
Guinea pig, Dunkin Hartley albino	20 M treated 10 control	Intradermal: 5 % (w/v) in acetone/alembicol Dermal: 80 % in acetone	Sensitising (M&K) [all animals sensitised] NeemAzal	Allan & Coleman, 1997a TOX9700507 OECD TG 406
Guinea pig, Dunkin Hartley albino	20 M treated 10 control	Intradermal: 0.5 % (w/v) in acetone/alembicol Dermal: 60 % in alembicol	Sensitising (M&K) [all animals sensitised] Fortune Aza	Allan & Coleman, 1997b TOX2005-2384 OECD TG 406
Guinea pig, Hartley albino	10 M treated 10 control	Dermal: 25 % (w/v) in ethanol	Sensitising (Buehler) [2/10 animals sensitised] NPI 720	Sherwood, 1990 TOX2005-2383 OECD TG 406

Table 2: CLP criteria for skin sensitisation

Guinea pig maximisation test (Magnusson Kligman Test)	Category 1A (H317): ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose
	Category 1B (H317): ≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
Buehler assay	Category 1A (H317): ≥ 15 % responding at ≤ 0.2 % topical induction dose or ≥ 60 % responding at > 0.2 % to ≤ 20 % topical induction dose
	Category 1B (H317): ≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

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

Number of animals (Guinea pigs)	Test substance Concentration of Azadirachtin	Doses product (Induction)	Doses active substance (Induction)	Result	Reference Report No Method
M&K 20 M treated 10 control	NeemAzal T/S 3% Azadirachtin Extract NeemAzal	Intradermal: 5 % in sesame oil Dermal: 100% undiluted	Intradermal: 0.15% Dermal: 4.5%	Not sensitising [no animal sensitised]	Kramer, 1998 981042830 OECD 406
Buehler 20 M treated 10 control	NeemAzal Formulation 4.5% Azadirachtin Extract NeemAzal	Dermal: 100% undiluted	Dermal: 4.5%	Not sensitising [no animals sensitised, 2/20 inconclusive]	Allan, Coleman, 1997c EIP 19/951048/SS OECD 406
Buehler 20 M treated 10 control	Fortune Aza 3% 26% Azadirachtin Extract Fortune Aza	Dermal: 100% undiluted	Dermal: 26%	Not sensitising [no animal sensitised]	Allan, Coleman, 1997d FBT 19/952235/SS OECD 406

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
Report: Kramer, H.-J. (1998)
 Skin Sensitation Study according to Magnusson & Kligman - NeemAzal T/S
 BioChem, Karlsruhe, Germany.
 Report-no. 981042830. TOX1999-224
Guidelines: OECD Guideline 406 (1992).
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, Borchon, 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 a undiluted 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
Report:	Allan, S., Coleman, D. (1997) NeemAzal Formulation - Skin Sensitisation in the Guinea Pig. Huntingdon Life Sciences Limited, England. Report-no. EIP 19/951048/SS. TOX9700521
Guidelines:	EPA FIFRA Guideline 152-115 (1984) 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.

Reference:	SPI IIIA 7.1.6
Report:	Allan, S., Coleman, D. (1997) Fortune Aza 3% EC - Skin Sensitation in the Guinea-Pig. Huntingdon Life Sciences Limited, England. Report-no. FBT 19/952235/SS. TOX2005-2482
Guidelines:	FIFRA 152-15 OECD Guideline 406 (1992).
Deviations:	None
GLP:	Yes (certified laboratory)
Acceptability:	The study is considered to be supplementary. (Buehler test with only 3 induction applications)

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

Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Owner
Allan, S., Coleman, D.	1997a	Neemazal technical skin sensitisation in the guinea-pig Huntingdon Life Sciences Ltd., Huntingdon, UK Trifolio-M GmbH Report-no. EIP 10/950818/SS GLP: yes Published: no	TRF
Allan, S., Coleman, D.	1997b	Fortune Aza technical Skin Sensitation in the Guinea Pig Huntingdon Life Sciences Ltd., Huntingdon, UK Oxon Italia S.p.A. Report-no. FBT 10/952234/SS GLP: yes Published: no	OXN
Allan, S., Coleman D.	1997c	NeemAzal Formulation skin sensitisation in the guinea-pig Huntingdon Life Sciences Ltd., Huntingdon, UK Trifolio-M GmbH Report-no. EIP 19/951048/SS GLP: yes Published: no	TRF
Allan, S., Coleman, D.	1997d	Fortune Aza 3% EC - Skin Sensitization to the Guinea-Pig Huntingdon Life Sciences Ltd., Huntingdon, UK Oxon Italia S.p.A. Report-no. FBT 19/952235/SS GLP: yes Published: no	OXN
Kramer, H.-J.	1998	Skin Sensitisation Study according to Magnusson & Kligman BioChem GmbH, Karlsruhe, Germany Trifolio-M GmbH Report-no. 981042830 GLP: yes Published: no	TRF
Sherwood, R.	1990	Dermal sensitization study of NPI 720 in guinea pigs using the modified Buehler method IIT Res. Inst. Life Scie.Operation, Chicago 60616-3799, USA Mitsui AgriScience International S.A./N.V. Report-no. L 08257 Study No 1 GLP: yes Published: no	MAS