Margosa, ext.
[from the kernels of *Azadirachta indica* extracted with water and further processed with organic solvents] = NeemAzal® technical

Evaluation of Classification and Labelling Proposal with regard to Developmental Toxicity

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- CONFIDENTIAL -

1 Executive Summary

Margosa, ext. [extracted with water] (NeemAzal® technical) is a refined medium polarity extract from the seed kernels of the Neem tree. It is approved in the EU as active substance for plant protection and biocidal products.

DS Germany prepared a CLH-Dossier for *Margosa, ext. [extracted with water]* and proposed classification of the technical material as skin sensitiser (Skin Sens. 1; H317) and as developmental toxicant (Repr. 2; H361d). The latter proposal was based on apparently increased incidences of interventricular septal defects, malrotated heart or supernumerary ribs, in the mid- and/or high dose groups of a developmental toxicity study in the rat conducted in 1997 according to OECD TG 414 and following GLP.

However, extended historical control data provided by Envigo the successor institute of the performing laboratory and included in the Appendix to this statement indicate that the low incidences of these findings are within the range of historical control data and are not substance induced.

In addition, a weight of evidence evaluation of the available data on *Margosa, ext. [extracted with water]* (NeemAzal® technical) and related Neem kernel extracts support the assessment that there is no indication that the findings which were noted only at maternally toxic dose levels are substance induced.

Overall, the reported findings do not warrant a classification of *Margosa, ext. [extracted with water]* (NeemAzal® technical) with regard to reproductive toxicity.

2 Summary

Margosa, ext. [extracted with water] (NeemAzal® technical) is a refined medium polarity extract from the seed kernels of the Neem tree. It is approved in the EU as active substance for plant protection (currently under the name *Azadirachtin*) and biocidal products (under the name *Margosa extract*)

During the plant protection approval process opinions were divided concerning the developmental toxicity of the active substance. In fact, a majority of experts agreed not to propose any classification based mainly on the low incidences observed in the developmental toxicity study in rats.

In 2018 DS Germany prepared a CLH-Dossier to define an appropriate harmonized classification for *Margosa, ext. [extracted with water]* and, regarding developmental toxicity, proposed classification in Category 2 (H361d).

With regard to developmental toxicity, the attention is focused on a teratogenicity study in the rat, especially on visceral malformations, namely the incidences of small and severe interventricular septal defect (VSD). Because incidences of VSD were very close to historical control data for the laboratory during the time period 1994-1995 (included in the report) and well within the published historical control data of MARTA (1996), it is appropriate to re-investigate this issue taking into account related relevant experimental data in order to assess these findings in a weight-of-evidence approach. The aim is to define a hazard classification that is both protective of human health and avoids an over-classification. Given the low incidence level of this rather common observation in rats, it is appropriate to consider further data and literature that are available:

a) Additional information on historical control data

In 2017 Trifolio-M GmbH asked for additional data from the performing lab, covering the time up to 1997. The additional data (provided in the Annex to this statement) demonstrate that the low incidences of adverse findings in the developmental toxicity study are well within the historical range of the performing laboratory of observations in control animals and are most probably not substance induced.

b) Studies on other registered Margosa extracts and published data from other Neem-kernel extracts

In 2014 one CLH dossier for three different Neem seed kernel extracts, summarized as "Azadirachtin", the active substance approved for the use in plant protection products, was submitted. The CLH dossier has been withdrawn in 2015 after it was decided that the substance identity has to be redefined (accompanied by a change of the name to “Margosa ext. + short description of the specific extraction process” for the a.i.’s in PPP). Still in case of the developmental toxicity it seems reasonable to take the data of other neem-based active substances into account. No indications of adverse developmental effects have been found in developmental studies from other notifiers. Five-batch analysis of the three extracts showed similarities in detectable components (11 were identified) and no

other possible adverse components were detected in *Margosa, ext. [extracted with water]*. The absence of adverse developmental effects can also be confirmed by published data from studies with other Neem-kernel extracts.

c) Literature on VSD and maternal toxicity

Treatment caused maternal toxicity in the dams, at both the mid-dose and high-dose group, noted as reduced body weight gain. Although this effect was only transient, it occurred around days 6 to 8 of pregnancy, which, according to literature (Fleeman et al., 2004, Purssell et al., 2012) has been identified as critical time in the development of the foetal heart. Furthermore, considering the outcome of the repeated dose studies, liver toxicity at dosages of 300 mg/kg bw can be expected. It is, therefore, reasonable to assume that the observed low incidence of VSD is a high dose effect secondary to maternal toxicity rather than a direct effect of *Margosa, ext. [extracted with water]*.

Therefore, all available data together with HCD and literature information should be considered using a **weight of evidence approach**. Basing any conclusion on single incidences at high dosage only , not exceeding historical control-range, in absence of statistical significance and without consideration of the biological significance, the influence of maternal toxicity and the consistency of the developmental findings (lack of similar observations in numerous other experimental studies on reproductive toxicity) should be avoided.

It is concluded that a classification of *Margosa, ext. [extracted with water]* for reproductive toxicity is not warranted supported by the following experimental findings:

- no indication of adverse developmental effects was noted in a two generation study (Ramamoorthy, 2000a)
- no adverse effects were reported in another teratogenicity study in the rat (Waterson, 1997c),
- no effects on the developing heart were reported in a teratogenicity study in rabbits (Ryan, 1994),
- no adverse effects were reported in supplemental studies including a segment I reproductive toxicity study, a segment II study and two 2-generation studies (Ramamoorthy, 2000b; Pugazhenthi, 1998; Ramamoorthy, 2000a; Mani, 1996),
- no adverse effects in pups were reported in a published 2-generation study which included detailed investigations on the developmental toxicity of Neem seed kernel extract (Dallaqua et al., 2013),
- no increased incidence of visceral malformations were reported in two published developmental toxicity studies on Neem seed kernel extracts (Srivastava & Raizada, 2001, 2007),
- the adverse effects are well within the historical control data

Based on the experimental evidence it is appropriate to not classify *Margosa, ext. [extracted with water]* with regard to reproductive toxicity.

Therefore, the classification and labelling according to Regulation (EC) No 1272/2008 with regard to developmental toxicity should be that *Margosa, ext. [extracted with water]* has not to be classified.

3 Introduction

Margosa, ext. [extracted with water] is a refined medium polarity extract from the kernels of the Neem tree. Different authorities have changed the name of the active substance (a.i.) in regulatory processes during the last years due to the difficulties to describe this botanical by means of suitable terms. In fact, since the patent application in 1990 the a.i. is known by customers under the name NeemAzal® technical - as claimed by the manufacturer. Under the name "Azadirachtin" the extract was included into Annex I of Directive 91/414/EEC by Commission 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 540/2011[1]. Under the name "Margosa Extract", it is approved as biocidal active

[1]). COMMISSION IMPLEMENTING REGULATION (EU) No 540/2011 of 25 May 2011, implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances

substance (PT 18)[2]. Margosa extract-based biocidal products have been endorsed by Authorities[3], and frequently and successfully applied to control certain pest insects (Oak Processionary Moth).

Margosa, ext. [extracted with water] is one of very few insecticidal active substances permitted in organic farming[4]. It has been used safely for 20 years as an active ingredient in several plant protection products (e.g. NeemAzal®-T/S).

4 CLH-Proposal - Reproduction Toxicity

In the CLH report 2018 (Proposal for Harmonised Classification and Labelling) the DS Germany concluded regarding classification and labelling that

- Regarding effects on fertility, the data are considered conclusive but not sufficient to trigger classification for such effects.
- Regarding developmental toxicity, classification in Category 2 (H361d, CLP criteria) is considered appropriate.
- No data are available to judge whether there are specific effects on or via lactation (R64, H362).

In contradiction to the classification in Category 2 (H361d, CLP criteria) the RMS noted in the CLH report:(4.10.4) "*Considering the findings seen in the developmental toxicity study in rats performed with NeemAzal (interventricular septal defects, malrotated heart, supernumerary ribs), the effects were seen at or around doses, where maternal toxicity could be observed. Additionally, the incidences in the rat study were increased only slightly and the possibility of non-specific causes such as general toxicity could not be excluded"*.

5 Available Data

5.1 Developmental Toxicity Study NeemAzal® (= *Margosa, ext. [extracted with water]*)

The discussed effects were noted in the following developmental toxicity study:

Report:	Myers, D. P., Dawe, I. S. (1997a) NeemAzal® technical – A Study of the Developmental Toxicity in Rats (Gavage administration) Huntingdon Life Sciences Ltd., Huntingdon, England unpublished report No. EIP 2/952493; TOX9700514

5.1.1 Executive Study Summary

In a GLP-conform study according to OECD TG 414 mated Charles River CD® female rats (Crl:CD®BR VAF/Plus strain), assigned to one control and three treatment groups of 25 animals each, were used to determine the teratogenic potential of *Margosa, ext. [extracted with water]* (NeemAzal® technical). Dosage levels of 50, 225 and 1000 mg/kg bw/d were administered orally by gavage on days 6 through 19 of gestation at a volume of 10 mL/kg in 1 % methylcellulose in this study. Observations on mortality, clinical signs of toxicity and body weights were recorded. On gestation day 20, all females were sacrificed, the number and location of viable and nonviable foetuses, early and late resorptions and *corpora lutea* were recorded. Uterus weights were determined. Gross lesions were recorded. Half of the foetuses from each dam were examined for skeletal deformations and the remaining were sectioned for identifying visceral anomalies.

[2] COMMISSION DIRECTIVE 2012/15/EU of 8 May 2012 amending Directive 98/8/EC of the European Parliament and of the Council to include margosa extract as an active substance in Annex I thereto

[3] Information an die Länder zur Bekämpfung des Eichenprozessionsspinners zum Schutz der menschlichen Gesundheit Dr. Kerstin Heesche-Wagner / BauA, 18.2.2013

[4] 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

Based on the initially reduced bodyweight and food consumption in the high and mid-dose groups the no observable adverse effect level (NOAEL) was 50 mg NeemAzal® /kg bw/d for maternal effects corresponding to 18.3 mg Azadirachtin A/kg bw/d.

One litter was affected by the mottled foetus syndrome and one by the squat foetus syndrome; both were discounted from further evaluation as these are considered spontaneous.

No effects on foetal number were observed.

An increase in the number of foetuses with supernumerary ribs was noted at the highest dose level but was not statistically significant. There were observations of visceral anomalies such as small interventricular defects.

Incidences of malformations affecting the heart of the foetuses were within the historical control data. Therefore, we conclude that there was no clear indication of an adverse developmental effect. The NOAEL was 1000 mg/kg bw/d.

5.1.2 Malformations - Low Number of Incidences

An important point for considerations on developmental toxicity is the number of malformations in dosage groups, compared to control data. For a proper judgement, malformations, which are considered as spontaneous (such as squat or mottled foetus syndrome) should be excluded from examination.

After exclusion of mottled foetus syndrome and squat foetus syndrome, only two malformations remain at the mid dose level and four malformations at the high dose level affecting one or three litters, respectively.

It should be noted that the cardiovascular malformations were to some extent clustered together in the same foetuses. Upon close re-examination it was only one foetus of 306 affected with malformations associated with the heart (one foetus in litter 63 was diagnosed with both, VSD and malrotated heart) in the mid dose group (Table 1).

Three foetuses of 308 in the high dose group were affected with malformations associated with the heart: One foetus (litter 88) was diagnosed with VSD, atrial septal defect and malrotated heart. One foetus (litter 80) was diagnosed with duplicated inferior vena cava and one foetus (litter 84) was diagnosed with duplicated inferior vena cava and VSD (Table 1).

Other visceral findings occurred only once in the mid dose or high dose group, and in most cases in combination with VSD, and are considered incidental observations. The observed increase of the number of foetuses showing supernumerary ribs was not statistically significant. This is in line with the statement of the study director on both the mid (225 mg a.i./kg bw) and high (1000 mg a.i./kg bw) dosage group: "*Neither the type, or incidence of skeletal anomalies, or the incidence of foetuses with sternebrae indicated any obvious adverse effects of treatment*."

Table 1: Foetal (litter) incidences of visceral malformations

Observation	Dose level (mg/kg bw/d)			
	0	**50**	**225**	**1000**
Number of foetus (litters) examined:	305 (23)	323 (23)	306 (23)	308 (23)
Interventricular septal defect + malrotated heart	0 (0)	0 (0)	1 (1)	0 (0)
Interventricular septal defect + duplicated inferior vena cava	0 (0)	0 (0)	0 (0)	1 (1)
Interventricular septal defect + malrotated heart + atrial septal defect with narrow pulmonary vein	0 (0)	0 (0)	0 (0)	1 (1)
Duplicated inferior vena cava	0 (0)	0 (0)	0 (0)	1 (1)

5.2 Interventricular Septal Defects

Interventricular septal defects (VSD) are rather commonly observed defects in the rat foetus (Solomon et al., 1997). Generally, these are categorised as "small" VSD which are considered to be a variation, a delay in cardiac development; these close postnatally without any adverse effect. The more severe VSD, which are considered as malformations are less common and would result in mortality (Fleeman et al., 2004).

5.2.1 Spontaneous Incidences /Historical Control Data

Both, small and severe VSD, occur spontaneously with apparently a genetic causal component as judged by the observation that in certain inbred strains of rats the incidence may be as high as 25 % in untreated control animals in the Wistar Kyoto rat (Slama et al., 2002).

Authorities focused on visceral malformations (interventricular septal defects, VSD) that showed an apparent dose response:

While only one of 306 foetuses in the mid dose group and three of 308 examined foetuses (three of 23 litters) were affected the incidence levels of the individual malformations were even lower with a maximum incidence of two affected foetuses or litters.

Considering historical control data (Table 2) it is demonstrated that these low incidences of malformations may be considered as spontaneous and not treatment-related findings:

HCD of the performing lab (1994-1995)

Historical control data from Huntingdon Life Sciences Ltd. (UK), the laboratory where the Myers & Dawe (1997a) study was conducted, were provided with the study report for 11 recent developmental studies conducted in July 1994 until February 1995 (as Appendix 12 to the study-report Myers & Dawe, 1997a). A maximum incidence of one affected pup was noted per study (0.63 %; 144 -176 pups examined per study) in the untreated control animals. Accordingly, only one litter was affected at maximum corresponding to 4.16 % of litters affected.

Additonal HCD of the performing lab (1994-1997) which have not yet been available to authorities:

In addition further historical data observed in developmental toxicity studies compiled from 24 studies conducted between July 1994 and February 1997 at Huntingdon Life Science Ltd. were provided by the legal successor institute Envigo (provided in the **Appendix** to this statement). These data demonstrate that VSD is occasionally observed in foetuses of untreated dams reported in five of 24 studies with a maximum of two cases (1.53 %) observed in two litters of one study in control animals.

Consequently, the incidences of one case of VSD in the mid dose group and two cases of VSD observed at high dose level (Myers & Dawe, 1997a) were within the range of the historical control values of the laboratory.

HCD Marta-study

The incidences in both groups (225 and 1000 mg a.i./kg bw) were also within the historical control data (1.34 %) published for Crl:CD®(SD)BR rat observed in a high number of laboratories (MARTA, 1996; Solomon et al., 1997).

Table 2 Interventricular septal defects (VSD) incidences and historical control maximum data

		Fetal data				Litter data	
		VSD malformation		VSD small anomaly		VSD malformation	VSD small anomaly
		No/no examined	%	No/no examined	%	%	%
Myers & Dawe, 1997	Control	0/152	0	0/152	0	0	0
	50 mg/kg bw/d	0/159	0	1/159	0.63	0	4.3 (1/23)
	225 mg/kg bw/d	1/152	0.66	3/152	1.97	4.3 (1/23)	13.0 (3/23)
	1000 mg/kg bw/d	2/151	1.32	2/151	1.32	8.7 (2/23)	8.7 (2/23)
HCD VSD (Huntingdon, UK, 07/94-02/95**)***		1/158	0.63	3/144	2.08	4.2 (1/24)	13.6 (3/22)
HCD VSD (Huntingdon, UK, 07/94-02/97**)***		2/131	1.53	3/132	2.27	8.3 (2/24)	13.6 (3/22)
HCD VSD membrane (Marta, 1996)*		-	-	-	10.3	-	40.9
HCD VSD muscular (Marta, 1996)*		unknown	1.34	-	-	10.0	-

*maximum incidence observed

Conclusion on comparison with historical control-data

Small VSD classified as anomaly were observed without clear dose response at incidences of one, three or two affected foetuses in the low, mid and high dose treatment group of the Myers & Dawe (1997a) study, respectively. In the historical control data it is evident, that small VSD are quite common, observed in control animals of 15 of the 24 studies, in fact are considered a retardation, as these small defects would close upon development of the foetus (Solomon et al., 1997). The incidence was up to three affected foetuses or three affected litters, the observed incidences in the study by Myers & Dawe (1997a) are within this range. This also matched with the historical control incidences reported in MARTA (1996).

Also, the combination of small and large VSD was noted in the control group of one study (study # 20, 6/96-Appendix) with both, two large VSD and two small VSD further supporting the view that the observed defects in the study by Myers & Dawe (1997a) are not substance induced.

Similarly, in a published compilation on studies using the Crl:CD®(SD)BR rat (MARTA, 1996) incidences of up to 10.3 % for ventricular septal defects of the membrane and of 1.34 % for muscular ventricular septal defects were reported. Therefore the observed incidences of this malformation of two affected foetuses of 151 (1.32 %) at high dose and one of 152 (0.66 %) in the concerned study with NeemAzal® can be considered well within the range of these historical controls. The incidence based on litters affected (2 of 23 = 8.7 %) is also well within the range reported with maximum values of 40.9 and 10.0 % for membrane and muscular VSD, respectively.

The low incidences of malrotated heart which were noted with one case in the mid dose group and one case in the high dose group are considered incidental, especially so as they were observed not as isolated malformation. Also the malformation “duplicated vena cava” was observed only in one foetus as an isolated malformation. This malformation is rarely observed in untreated controls, but was also seen in the historical control data of developmental toxicity studies conducted between July 1994 and February 1997 at Huntingdon Life Science Ltd.

5.2.2 Chemically Induced VSD

Increased incidences of VSD have been reported for rats following treatment of pregnant animals with certain chemical substances, such as the anti-convulsant trimethadione, dimethadione or trichloroethylene. These have been reported to induce rates of up to 93 % incidence of VSD. Purssell et al. (2012) noted that although these chemicals are structurally diverse a common denominator appears to be the induction of toxicity affecting the heart and cardiovascular effects. No such effects, however, were noted for *Margosa, ext. [extracted with water]*.

Ventricular septal defects are observed rather commonly as spontaneous anomalies regarded as delayed development or visceral malformation. Also, certain chemical substances including anti-epileptic drugs and organic solvents have been reported to induce an increased incidence of VSD. The low incidence of VSD and other observations reported in the Myers & Dawe (1997a) study are within the historical control background incidences. Thus, it appears not appropriate to translate this finding into a classification of *Margosa, ext. [extracted with water]* with regard to a developmental toxicological hazard.

5.3 Skeletal variations

An apparent increased number of foetuses with a short 14th rib was observed in the study by Myers & Dawe (1997a) for the high dose group with 35 affected foetuses (35 of 149 examined equals 23.5 % of the foetuses examined – there is a typo in the table 9 of the study report, reproduced here as Table 3 below). However, there was no statistical significance and, when total skeletal variants were considered there was no adverse effect noted.

Furthermore historical control data from the laboratory Huntingdon Life Sciences Ltd., UK (07/1994 to 02/1997) show that up to 37 (21.3 %) foetuses with 14th rib were noted in untreated control animals with up to 52 % of litters affected. The observation of 35 affected foetuses (23.5 %, no information of litter incidence) in the high dose group of the NeemAzal®-study is regarded as within the range of historical control data and just above the range of percentages

Table 3 Skeletal variants group values (Table 9 of the study report)

Skeletal variants - group values

Group/ dosage (mg/kg/day)	Foetuses examined#	Foetuses with													
		13 Ribs		14 Ribs		Normal sternebrae		Unossified sternebrae		Inc. Oss sternebrae		Misa./Bip. sternebrae		Total Variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1 Control	152	137	90.6	15	9.4	75	47.7	38	27.0	47	30.6	6	4.6	77	52.3
2 50	159	145	91.4	14	8.6	92	59.0	31	18.3	45	27.7	3	2.0	67	41.0
3 225	149	138	93.3	11	6.7	86	57.9	25	17.3	48	32.7	6	4.2	63	42.1
4 1000	149	114	75.7	35	24.3	77	51.0	32	22.4	50	33.3	4	2.6	72	49.0

Excludes malformed foetuses
Inc. Oss. Incomplete ossification
Misa. Misaligned
Bip Bipartite
Statistical analyses of % affected: No statistical significance (p >0.05)

5.4 Toxicity

5.4.1 Maternal Toxicity

OECD TG 414 for prenatal developmental toxicity studies recommends: "*The highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering. At least one intermediate dose level should produce minimal observable toxic*

effects."[5] Therefore, in tests with substances of low toxicity, a very high dose must be applied to produce any toxicity at all. Hence the highest dose in the development study with *Margosa, ext. [extracted with water]* had been set to 1000 mg/kg bw in order to obtain maternal toxicity. Even at the mid dose (225 mg/kg bw), slight maternal effects (decreased food consumption, post dosing salivation) were noted. Undisputedly, maternal toxicity was observed at the highest dose level of 1000 mg/kg bw/d (Table 4).

In the mid dose group (225 mg/kg bw/d) a significantly reduced feed intake (days 6-7) and a reduced body weight gain (days 6-8; 8.5 g vs 10.5 g (-19%), p=0.18) was observed when all pregnant dams are considered. This reduction in body weight gain by 19 % is only a transient and not-statistically significant sign of maternal toxicity. However, it is just below the value of 20 % decreased body weight gain mentioned in a recent review on the effects of maternal toxicity in developmental toxicity studies (Beyer et al., 2011). Thus, in conclusion, maternal toxicity has to be considered at mid dose level.

Table 4: Maternal body weight changes and feed intake

	Dose Level			
NeemAzal technical [mg/kg bw/d]	**0**	**50**	**225**	**1000**
Azadirachtin A [mg/kg bw/d]	**0**	**18.3**	**82.4**	**366**
Number of animals§	23	23	23	23
Body weight gain				
Weight gain Day 6 – Day 8 [g/rat]	10.4	10.5	8.5#	6.1**
Weight gain Day 8 – Day 20 [g/rat]	133.1	143.8	138.7	143.0
Feed intake				
Number of animals§	25	25	25	25
Feed intake Days 6 – 7 [g/rat/d]	26	26	24**	23**
Feed intake Days 8 – 9 [g/rat/d]	28	29	28	27
Feed intake Days 10 – 11 [g/rat/d]	30	31	29	28*
Feed intake Days 12 – 13 [g/rat/d]	30	29	29	27

#p = 0.18
**p < 0.01, * p < 0.05
§ excluding non-pregnant animals for body weight (two per dose group)

Interventricular septal defect (VSD) is a relatively common anomaly and malformation in laboratory animals and is noted also in untreated control groups (see Point 4.2.1). It has been reported to may have a genetic basis as evidenced by the observation that the rate of VSD is high in inbred rat strains (Solomon et al., 1997). In addition, chemical inducers have been identified such as the anticonvulsant trimethadione or phenobarbital. These have been reported to induce rates of up to 93 % incidence of VSD and are most effective in the induction of these defects when administered around gestation day 8 (Fleeman et al., 2004, Purssell et al., 2012). Thus, the observed transient toxicity in the mid dose group (manifest as reduced feed intake and reduced body weight gain) was during that very period of the foetal development that is important for the organogenesis also of the heart.

5.4.2 Toxicity of *Margosa, ext. [extracted with water]* in the Rat upon Repeated Oral Administration

As mentioned in paragraph 40 of OECD 414 the study results need to be interpreted in connection with subchronic studies:

"A prenatal developmental toxicity study will provide information on the effects of repeated oral exposure to a substance during pregnancy. The results of the study should be interpreted in conjunction with the findings of subchronic, reproduction, toxicokinetic and other studies. Since emphasis is placed on both general toxicity and developmental toxicity endpoints, the result of the study will allow for the discrimination between developmental effects occurring in the absence of general toxicity and those which are only expressed at levels that are also toxic to the maternal animal".

[5] OECD (2018): Guideline for testing of chemicals. Prenatal developmental toxicity study (No. 414); Organisation for Economic Cooperation and Development, Paris

Therefore, it seems reasonable to have a proper look on the lowest observed adverse effect levels (LOAEL) of repeated dose studies on oral toxicity in the rat.

Table 5: Lowest observed adverse effect levels in repeated dose studies on oral toxicity in the rat for determination of maternal toxicity (as summarised in the DAR)

Study type	Test item	LOAEL Margosa ext. (a.i.) (mg/kg bw/d)	LOAEL Azadirachtin A (mg/kg bw/d)	Report
28-day toxicity	NeemAzal	301*	83.1	Waterson (1997a)
28-day toxicity	Fortune Aza	400	40.8	Waterson, Dawe (1997a)
90-day toxicity	NeemAzal	123	33.9	Waterson (1997b)
90-day toxicity	Fortune Aza	137	14.0	Waterson, Dawe (1997b)
90-day toxicity	ATI-720	178	13.8	Johnson (1994)
Prelim teratogenicity	Fortune Aza	>1000	100	Waterson (1997d)
Teratogenicity	Fortune Aza	>1000	100	Waterson (1997c)
Prelim teratogenicity	NeemAzal	300	108	Myers, Dawe (1997b)
Teratogenicity	NeemAzal	1000	360	Myers, Dawe (1997a)#

*lowest dose level tested, no NOAEL in this study
study discussed here

Comparing the lowest adverse effect levels observed in rats upon repeated oral administration (Table 5) the range is between 123 and >1000 mg a.i./kg bw/d for the tested Neem-kernel extracts (corresponding to 33.9-360 mg Azadirachtin A). In the range finding study with NeemAzal® (Myers & Dawe, 1997b) clear signs for maternal toxicity occurred at the mid dose level and the LOAEL was set to 300 mg/kg bw/d (**NOAEL: 100 mg/kg bw/d**) as cited in 4.10.2.1 (table 34) of the current CLH report 2018.

The lowest limit values (LOAEL 123 – 178 mg a.i./kg bw/d) were recorded for administration with feed whereas bolus administration by gavage, as in the teratogenicity study, is known to generally lead to more pronounced effects at lower levels.

In the subchronic repeat dose toxicity studies (90-day-toxicity) performed with the technical products, signs of toxicity were seen. Thus mentioned in the DAR[6]:

The NOAELs of the different extracts in the subchronic studies were 32 and 35 mg a.i./kg bw/d. Based on these, an overall NOAEL of 32 mg a.i. /kg bw/d (corresponding to 32 mg NeemAzal® technical) was defined in the plant protection approval process. This was the base for the limit values AOEL = ADI = 0.1 mg a.i./kg bw/d including a safety factor of 300[7].

Thus, the lowest LOAEL observed was 123 mg a.i./kg bw/d. This value corresponds to about half of the mid dose level of 225 mg a.i./kg bw/d in the teratogenicity study with NeemAzal, which we consider to be the LOAEL for maternal toxicity.

While this aspect is not addressed in the current CLH-report, it was part of the CLH-evaluation 2014 (9.10.2.1)[8]: "*Even though maternal toxicity was not observed in this study, liver toxicity in dams can be expected, which had a LOAEL of 123 mg NeemAzal/kg bw/d (1600 ppm) in the 90-d study in rats (NOAEL: 32 mg/kg bw/d (400 ppm)).*"

Based on these considerations it appears reasonable to consider the mid dose level of 225 mg a.i./kg bw/d as LOAEL for maternal toxicity in the Myers & Dawe (1997a) study.

[6] RMS Germany (2008) Azadirachtin DAR 06 Annex B.6 toxicology and metabolism p.202-214.

[7] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance azadirachtin, List of endpoints EFSA Journal 2011;9(3):1858. [76 pp.]. doi:10.2903/j.efsa.2011.1858.

[8] BAuA, 2014; Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2, substance name Margosa, ext.

5.5 Developmental Toxicity study in the Rat with Fortune Aza

Based on the data submitted, it was concluded in the process for approval of Azadirachtin (=*Margosa, ext. [extracted with water]*) as active substance for plant protection that three Azadirachtin- (Margosa-) extracts (NeemAzal®, Fortune Aza and Mitsui extract) are equivalent. Although it was decided that the substance identity has to be redefined, the extracts of the other taskforce members could still be used as additional information. Five-batch analysis of the three extracts showed similarities in detectable components (11 were identified) and no possibly adverse other components were detected in *Margosa, ext. [extracted with water]*. Thus, the study on teratogenicity in the rat conducted with Fortune Aza should be regarded as additional study on an equivalent test item.

This GLP-conform study according to OECD TG 414 was conducted at the same laboratory (Huntingdon, UK) by the same personnel (foetal pathologist), in the same year (1997) with the same animal strain (Crl:CD®BR VAF/Plus) from the same source (Charles River UK) as the study with NeemAzal® (Myers & Dawe, 1997a).

Report:	Waterson, L.A. (1997c) Fortune Aza technical - A Study of the Developmental Toxicity in Rats, Huntingdon Life Sciences Ltd., Huntingdon, England unpublished report No. FBT 2/960340

This study demonstrated a lack of adverse effects on foetuses following administration of Margosa extract during pregnancy at dose levels of 100, 300 or 1000 mg/kg bw/d as concluded by the RMS. In fact the number of interventricular septal defects was highest in the control group with three animals from three litters showing small VSD while one foetus was affected in the mid-dose group. No malformations affecting the heart or blood vessels or any other kind of malformations were noted.

5.6 Other Data

In a weight of evidence evaluation it is appropriate to consider further available data on *Margosa, ext. [extracted with water]* (NeemAzal® technical) and related Neem kernel extracts.

5.6.1 Human Evidence

No epidemiological studies have been reported indicating an adverse effect of *Margosa, ext. [extracted with water]* on development. Furthermore, there are no casuistic reports on teratogenic or other adverse developmental effects in humans despite the common use of Neem-kernel-products.
As pointed out in the CLH report (Point 4.10.2.2.) there are numerous published reports on the use of extracts from other parts of the Neem tree (leaves, bark, roots), or Neem oil in Indian folk medicine. Because of a marked different composition, these have no relevance for the refined *Margosa, ext. [extracted with water]* that is considered here.

5.6.2 Reproduction Toxicity Studies

Additional information is available that was disregarded in the process for approval of Azadirachtin (Margosa ext.) as active substance for plant protection (PPP process), but can very well serve at least as supportive and supplemental information for the classification of *Margosa, ext. [extracted with water]* according to Regulation (EC) No. 1272/2008.

Malformations such as severe VSD lead to the death of affected offspring. Thus, reduced survival in reproductive studies would be indicative of increased incidences of severe malformations. However, no increase in mortality in pups was reported in a two generation study (Ramamoorthy, 2000a) at dose levels of up to 750 ppm NeemAzal® technical in the diet. This study was accepted in the DAR[9] and the CLH report.

Report:	Ramamoorthy (2000a) Evaluation of Toxicity of NeemAzal Technical to Reproductive Process in Wistar Rats - Segment IV - Toxicity to two Generation Reproductive Process, Report-no. 4826,

Additionally, reports on three further generational studies are available that were disregarded in the PPP process and judged "not acceptable" in the CLH report. However, these can serve at least as supportive and supplemental information for the classification of *Margosa, ext. [extracted with water]* despite considerable deficiencies in reporting and/or study design or the test substance information.

These include a two generation reproduction study with the plant protection product NeemAzal F 5% (Mani, 1996). No adverse effects induced by this treatment were observed in the offspring regarding clinical signs, body weight, sex ratio and litter size in F1a and F1b pups, or F2a and F2b pups.
No lesions were noted in F2b pups that were subjected to necropsy, neither with regard to gross pathology nor to histopathological examinations performed on control animals and in the group with the highest dose.
Reproductive performance and other litter parameters were not affected by the treated diet at any dose.

In a segment I study (Ramamoorthy, 2000b) rats received NeemAzal® at 100 and 1000 mg/kg bw/d by gavage. There were no treatment related developmental effects reported regarding litter size, fertility, pup weight or any other signs in the offspring. The NOAEL was 1000 mg NeemAzal®/kg bw/d. Although this study was not accepted in the CLH report it points out that even at a dosage of 1000 mg NeemAzal®/kg bw/d no effects on general reproduction parameters were determined.

In a published two generation study the absence of developmental toxicity of Azadirachtin (technical Azadirachtin 12 % = Margosa extract) was demonstrated (Srivastava & Raizada, 2007):
Rats were fed 100, 500 and 1000 ppm technical Azadirachtin through diet, which is equivalent to 5, 25 and 50 mg/kg bw of rats. There were no toxicological effects in parent rats as evidenced by clinical signs of toxicity. The litters of F(1B) and F(2B) generations were devoid of any morphological, visceral and teratogenic changes. There were no major malformations in foetuses, while some insignificant minor skeletal variations like missing 5th sternebrae and bipartite thoracic centre were found. These were not compound or dose related. No significant pathomorphological changes were observed in liver, kidney, brain and gonads of F(2B) pups.
In conclusion, rats fed technical Azadirachtin (= Margosa extract) showed no evidence of cumulative effects on postnatal development and reproductive performance over two generations.
The lack of teratogenic potential is judged by the observation that offspring mortality was unaffected and also no teratogenic effects were observed in a subgroup of foetuses investigated for skeletal or visceral malformations.
The latter report was disregarded in the DAR (based on non-equivalence of the test item) and not mentioned in the CLH report. However, this published study adds to the database on Margosa extract/Azadirachtin supporting the notion that the malformations reported in the Myers & Dawe (1997a) study are a spontaneous and incidental finding.

5.6.3 Developmental Toxicity Studies

Further evidence for the observation of maternal toxicity of technical Neem-kernel extract at, or around 200-300 mg a.i./kg bw/d and the lack of any developmental toxicity is given in the dose range finding studies for the full studies (detailed in 4.1 and 4.2) and a developmental toxicity study (Segment II study):

Study 1) A GLP-conform dose finding teratogenicity study was performed in mated Charles River CD® rats according to OECD guideline 414 (Myers & Dawe, 1997b). One control and three treatment groups of 10 animals, each, were used to determine the teratogenic potential of NeemAzal® technical. Dosage levels of 0, 100, 300 and 1000 mg/kg bw/d were administered orally by gavage on days 6 through 19.
Observations on mortality, clinical signs of toxicity and body weights were recorded. On gestation day 20, all females were sacrificed and the number and location of viable and nonviable foetuses, early and late resorptions and *corpora lutea* were recorded. Uterus weights were determined. Gross lesions were recorded.
Maternal toxicity: The post dose salivation observed for dams at 300 and 1000 mg/kg bw/d is a common observation in studies employing the oral gavage route and is possibly a reaction to the bitter taste of the test substance.
Based on the initially reduced bodyweight gain (Table 5) and food consumption and the increased water consumption in the high dose group, the no observable adverse effect level (NOAEL) for maternal toxicity in this preliminary study was set at 100 mg/kg bw/d corresponding to 36.6 mg Azadirachtin A/kg bw/d.
Developmental toxicity: No effects on foetal number and development or incidences of malformations were observed at any treatment levels. Thus, the NOAEL for developmental toxicity was >1000 mg/kg bw/d.

Table 5: Maternal body weight changes in dose finding teratogenicity study (excerpt from Myers & Dawe, 1997b)

	Dose level [mg/kg bw/d]			
NeemAzal® technical	**0**	**100**	**300**	**1000**
Azadirachtin A	**0**	**36.6**	**109.8**	**366.0**
Number of animals	9	8	8	8
Weight gain Day 2-Day 6 (g/rat)	42.6	39.6	29.6	42.6
Weight gain Day 6-Day 8 (g/rat)	14.2	13.8	8.8*	8.6*
Weight gain Day 8-Day 10 (g/rat)	14.7	15.1	17.9	19.0
Weight gain Day 10-Day 20 (g/rat	116.0	114.3	118.0	117.6
Final bodyweight (g/rat)	410.1	406.5	409.3	413.0

* $p<0.01$

Study 2) In a GLP-conform dose range finding study (Waterson, 1997d) according to OECD guideline 414 (1981) mated Charles River CD® female rats, assigned to one control and three treatment groups of 10 animals, each, were used to determine the teratogenic potential of Fortune Aza technical. Dosage levels of 0, 100, 300 and 1000 mg/kg bw/d were administered orally by gavage on days 6 through 19 of gestation at a volume of 10 mL/kg in 1 % methylcellulose in this study. Observations on mortality, clinical signs of toxicity and body weights were recorded. On gestation day 20, all females were sacrificed and the number and location of viable and nonviable foetuses, early and late resorptions and *corpora lutea* were recorded. Uterus weights were determined. Gross lesions were recorded.
Based on the initially reduced bodyweight and food consumption, and the increased water consumption in the high dose group the NOAEL was 300 mg/kg bw/d for maternal toxicity (19.8 mg Azadirachtin A/kg bw/d).
The post dose salivation observed for dams at 300 and 1000 mg/kg bw/d is a common observation in studies employing the oral gavage route and is possibly a reaction to the bitter taste of the test substance.
No effects on foetal number and development or incidences of malformations were observed at any treatment levels. Thus, the NOEL for developmental toxicity was 1000 mg/kg bw/d.

Study 3) In a segment II study (Pugazhenthi, 1998) groups of 20 pregnant female Wistar rats were used to determine the teratogenic potential of NeemAzal®. This study was not performed according to GLP but adhered closely to a similar quality system.
Dosage levels of 0, 250, 500 and 1000 mg/kg bw/d were administered orally by gavage on days 6 through 15 of gestation at a volume of 10 mL/kg in distilled water in this study.
Based on reduced uterus weight and reduced food consumption in the high dose group the NOAEL was 500 mg/kg bw/d for maternal effects.
Only increased incidences of moles were noted in the foetuses of the high treatment groups. No other effects on foetal number and development were observed. No VSD were reported.
The latter report was not evaluated in the CLH report due to reporting deficits but adds further to the evidence that malformations reported in the Myers & Dawe (1997a) study are a spontaneous and incidental finding.

Study 4) In a GLP-conform teratogenicity study in the rabbit (Ryan, 1994) according to OECD guideline 414 (1981) four groups of pregnant New Zealand White rabbits were treated daily on gestation days 6 to 18 by gavage. The animals received suspensions of Margosa extract ATI-720 in 0.5 % aqueous carboxymethyl cellulose at 20, 100 and 500 mg/kg bw/d, and a control group was treated with vehicle alone (5 mL/kg bw).

Based on bloody urine in the high dose group, and reduced bodyweight and food consumption in the high dose and mid dose group the NOAEL was 20 mg ATI-720/kg bw/d for maternal effects.
Significant signs of developmental toxicity were observed in the high dose group only and were related to maternal toxicity. No effects on foetal number and development were observed in the mid dose and low dose group. Thus, the NOAEL for developmental toxicity was 100 mg/kg bw/d. No visceral malformations affecting the heart were observed. This study was conducted in rabbits but may be considered to add further to the evidence that malformations affecting the heart reported in the Myers & Dawe (1997a) study are a spontaneous and incidental finding.

Study 5) Most recently Dallaqua et al. (2013) compared Margosa extract (extract of Neem seed kernels with 70 % aqueous ethanol) and Neem seed kernel oil in a developmental toxicity study in Sprague-Dawley rats. The study was not conducted under GLP, and deviations from the OECD guideline 414 included that only one dose level (1.4 g/L) and a control group was tested. In each group only 11 dams were included and thus only 39 pups were tested for visceral malformations and 43 for skeletal malformations. In conclusion, while Neem seed kernel oil

treatment administered during pregnancy caused abnormalities in rat foetuses, showing teratogenic effect, the Margosa extract (ethanolic extract) presented no impairment in the foetuses.

Considering the deficiencies and deviations from the relevant guideline, this study may only be considered as supplemental, but it adds to the evidence that Neem seed kernel=Margosa extract does not possess a teratogenic potential.

Study 6) In a published teratogenic study (Srivastava & Raizada, 2001) the absence of developmental toxicity of Margosa extract (technical Azadirachtin 12 %) was demonstrated. Rats were fed with 500, 1000 and 1500 mg/kg bw/d technical Azadirachtin through diet. Technical Azadirachtin at different doses did not produce any significant embryo/foetotoxic effects. There were no major malformations, while some minor variants found in animals at the high doses were not compound or dose related effects. The absence of anomalies in foetal gross, visceral morphology and skeleton suggests that technical Azadirachtin is not teratogenic in rats at the doses tested. Even if the equivalence of the extracts is not fulfilled, this published study adds to the database on Margosa extract/ Neem seed kernel extract supporting the notion that the malformations reported in the Myers & Dawe (1997a) study are a spontaneous and incidental finding.

Table 6 Summary of the weight of evidence regarding developmental toxicity of Margosa extract

Study type **exposure route** **species**	**Guideline followed** **Test item**	**Developmental toxicity effects**	**Reference**
Developmental toxicity; oral gavage, rat	OECD TG 414 NeemAzal	Adverse effects within the published historical control range	Myers & Dawe, 1997a
Developmental toxicity; oral gavage, rat	OECD TG 414 Fortune Aza	No adverse effect	Waterson, 1997c
2-generation oral (with feed), rat	Gaitonde Guideline NeemAzal	No adverse effects	Ramamoorthy, 2000a
2-generation & developmental toxicity oral (with feed), rat	Published No guideline mentioned	No adverse effects	Srivastava & Raizada, 2007
2-generation oral (with feed), rat	Gaitonde Guideline NeemAzal F 5%	No adverse effects	Mani, 1996
Segment I study oral gavage, rat	Gaitonde Guideline NeemAzal	No adverse effects	Ramamoorthy, 2000b
Developmental toxicity; oral gavage, rat	Published No guideline mentioned	No adverse effects	Srivastava & Raizada, 2001
Developmental toxicity; oral gavage, rat	OECD TG 414 DRF NeemAzal	No adverse effect	Myers & Dawe, 1997b
Developmental toxicity; oral gavage, rat	OECD TG 414 DRF Fortune Aza	No adverse effect	Waterson, 1997d
Segment II study oral gavage, rat	Gaitonde Guideline NeemAzal	No adverse effect	Pugazhenthi, 1998
Developmental toxicity; oral gavage, rabbit	OECD TG 414 ATI 720	No visceral findings Effects only at severe maternal toxic dose levels	Ryan, 1994
Developmental toxicity; oral gavage, rat	Non guideline 70% ethanolic extract	No adverse effect	Dallaqua et al., 2013

6 Opinion of PRAPeR Meeting

EFSA organised a peer review of the initial DAR-evaluation. During the PRAPeR expert meeting 79 (section mammalian toxicity) the developmental toxicity was discussed explicitly. The most important conclusion of this expert meeting is cited in the draft CLH report (Chapter 4.11.5):

"During an expert consultation in the PPP procedure, it was discussed, whether classification with R63 should be proposed: "There was a feeling that R63 was not appropriate based on the dataset available and incidences seen in the rat studies. […] Experts voted on the classification issue and a majority agreed to not propose any classification" (cited from the meeting minutes). This recommendation was based mainly on the low incidences observed in the developmental toxicity study in rats with NeemAzal."

7 EFSA Conclusion on Pesticide Peer review

After the PRAPeR expert meetings, EFSA published its conclusion on pesticide peer review with the following statement on developmental effects[10]:

„No developmental effects were observed in rats with Trifolio-M and Sipcam sources…..“

8 Discussion

Low incidences of malformations were noted in a rat teratogenicity toxicity study with one case of ventricular septal defect in the mid dose treatment group and two pups in the high dose group (within the historical control range of the laboratory) suggestive of a dose-related effect. This malformation is a rather common spontaneous observation in rat foetuses (Solomon et al., 1997). Mostly these VSD are categorised as “small” and are considered to be a variation, a delay in cardiac development; these close postnatally without any adverse consequences for the affected foetus. While the VSD categorised as "large" are severe and considered a malformation, which is less common and result in mortality (Fleeman et al., 2004). Increased incidences of VSD have been observed in rat foetuses following treatment of pregnant animals with certain chemical substances. A common denominator appears to be the induction of toxicity affecting the heart and cardiovascular effects (Purssell et al., 2012). No such effects, however, were noted for *Margosa, ext. [extracted with water]* in any of the toxicity studies, the heart and cardiovascular system is not a target organ of *Margosa, ext. [extracted with water]* induced toxicity as summarised in the DAR[i] and CLH report.

Furthermore, the treatment caused maternal toxicity in the dams both at mid dose and high dose level noted as reduced body weight gain. Although this effect was only transient, it occurred around days 6 to 8 of pregnancy, which has been identified as critical time in the development of the foetal heart. Furthermore, toxicity noted upon repeated dosing was reported at 123 mg/kg bw/d corresponding to half the mid dose level of 225 mg/kg bw/d.

Short supernumerary ribs are considered variations rather than malformations and do not trigger classification for reproductive toxicity. The observed increases in incidence rates were not statistically significant and are within the historical control data.

Overall, there was only one pup in the mid dose group identified with visceral malformations, and only three foetuses (three litters) affected in the high dose in the Myers & Dawe (1997a) study. These low incidences are within the historical control range.

Considering the weight of evidence there are no data supporting a classification of *Margosa, ext. [extracted with water]* for reproductive toxicity:

No indication of adverse developmental effects were noted in a two generation study, also no adverse effects were reported in other teratogenicity studies with Margosa extracts with rats, no effects on the developing heart were reported in a teratogenicity study in rabbits. No adverse effects were reported in supplemental studies including a segment I reproductive toxicity study and a two generation study. No adverse effects in pups were reported in a published two generation study, which included detailed investigations on teratogenicity of Margosa extract.

9 Conclusion

Ventricular septal defects (VSD, small and large) are observed rather commonly as spontaneous anomalies regarded as either delayed development (small VSD) or visceral malformation (large VSD). The low incidence of VSD and other observations reported in the study by Myers & Dawe (1997a) are not statistically significant, within the historical control background incidences and have occurred in the presence of other toxic effects. It should be noted that the cardiovascular malformations were to some extent clustered together in the same foetuses. Furthermore, the specific cardiovascular malformation seen following treatment with *Margosa, ext. [extracted with water]* was not reported consistently in 11 other studies with Margosa extracts on teratogenicity. It can be concluded that the overall evidence is insufficient for classification because the findings seen (at low incidences) were either likely to be due to maternal toxicity and/or could be considered as chance findings.

[10] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance azadirachtin. EFSA Journal 2011;9(3):1858. [76 pp.]. doi:10.2903/j.efsa.2011.1858

Thus, it is not appropriate to translate the findings in the Myers & Dawe (1997a) study into a classification of *Margosa, ext. [extracted with water]* (Azadirachtin/NeemAzal®) with regard to a developmental toxicological hazard.

The proposed classification and labelling according to Regulation (EC) No 1272/2008 with regard to human health is therefore only: H317 May cause an allergic skin reaction.

10 References

10.1 Proprietary studies

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Mani, B. (1996) Reproduction Toxicity Study (Segment-IV) of NeemAzal-F 5% in Charles Foster Rat, Report-no. 1542/JRF/TOX/96, GLP: yes,

Myers, D.P., Dawe, I.S. (1997a) A study of developmental toxicity in rats (gavage administration) Report-no. 05-293-97, EIP 2/952493, GLP: yes,

Myers, D.P., Dawe, I.S. (1997b) NeemAzal technical - A preliminary study of developmental toxicity in rats (gavage administration) Report-no. EIP 2/952493, GLP: yes,

Pugazhenthi, R. (1998) Effect of NeemAzal technical 27.3% on reproductive processes: Segment II teratological study in wistar rats, Report-no. 4824,GLP/GEP: no,

Ramamoorthy, S. (2000a) Evaluation of Toxicity of NeemAzal Technical to Reproductive Process in Wistar Rats - Segment IV - Toxicity to two Generation Reproductive Process, Report-no. 4826, GLP: yes,

Ramamoorthy, S. (2000b) Evaluation of toxicity of NeemAzal technical to general reproductive process and fertility in Wistar rats - Segment I, Report-no. 4823, GLP/GEP: no

Ryan, B. (1994) A developmental toxicity study of orally administered ATI-720 in rabbits, Report-no. 129601, L 08424 Study o2b, GLP: yes,

Waterson, L.A. (1997a) NeemAzal technical toxicity study in rats by dietary administration for 4 weeks, Report-no. EIP 3/960397, GLP: yes,

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10.2 Published data

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Srivastava, M.K., & Raizada, R.B. (2007) Lack of toxic effect of technical azadirachtin during postnatal development of rats. *Food and chemical toxicology*, 45(3), 465-471.

Appendix

Provided by D. Myers, Envigo CRS Ltd., Huntingdon, UK, 2017

Control Incidence Major Abnormalities and Minor Skeletal/ Minor Visceral Abnormalities - Crl:CD(SD) Rat

Study number#	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**		**13**		**14**		**15**		**16**		**17**		**18**		**19**		**20**		**21**		**22**		**23**		**24**		**Min**	**Max**	**Min**	**Max**
Study type	Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Main		Foetus		Litter	
Necropsy start date	7.94		7.94		9.94		9.94		10.94		11.94		11.94		1.95		1.95		2.95		2.95		10.95		11.95		2.96		3.96		3.96		4.96		5.96		5.96		6.96		8.96		11.96		12.96		2.97					
Major Abnormalities - Malformations																																																						
Number fetuses/litters examined	287	22	292	22	289	22	323	24	319	24	327	24	343	25	281	23	294	24	324	23	307	24	263	20	310	25	325	24	258	20	281	24	273	23	314	25	269	22	269	24	350	25	284	22	232	18	298	24				
Number fetuses/litters examined at necropsy	144	22	146	22	144	22	161	24	158	24	164	24	171	25	139	23	147	24	160	23	156	24	132	20	156	25	161	24	128	20	139	24	136	23	157	25	134	22	131	24	176	25	143	22	113	18	146	24				
Cervical/Thoracic																																																						
Malrotated heart	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
Ventricular septal defect	-	-	-	-	-	-	-	-	1	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	2	2	-	-	-	-	1	1	-	-	0	2	0	2
Duplicated vena cava	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	0	1
Minor skeletal Abnormalities																																																						
Number fetuses/litters examined	143	22	142	22	145	22	159	24	159	24	161	24	169	25	140	23	147	24	159	23	151	24	131	20	152	25	160	24	129	20	142	24	136	23	155	25	135	22	130	24	174	25	141	22	116	18	152	24				
14th rib																																																						
full supernumerary	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	0	2	0	1
short supernumerary	19	11	24	12	18	11	17	8	21	11	21	12	19	10	16	9	14	7	13	5	15	8	6	*	13	12	11	7	10	5	11	7	13	8	15	8	14	10	11	5	35	12	18	9	18	9	21	14	6	35	5	12
%	13.8		17.1		12.3		10.5		13.0		12.3		11.1		10.1		8.8		7.5		9.0		4.5		8.5		6.9		7.7		10.5		10.4		10.3		9.8		7.2		21.3		12.8		15.2		13.4		4.5	21.3		
Minor visceral Abnormalities																																																						
Number fetuses/litters examined at necropsy	144	22	146	22	144	22	161	24	158	24	164	24	171	25	139	23	147	24	160	23	156	24	132	20	156	25	161	24	128	20	139	24	136	23	157	25	134	22	131	24	176	25	143	22	113	18	146	24				
Cervical/Thoracic																																																						
small ventricular septal defect	3	3	-	-	-	-	1	1	-	-	-	-	2	2	2	2	1	1	2	2	1	1	3	2	3	3	-	-	-	-	-	-	3	3	1	1	2	2	2	2	-	-	-	-	2	2	2	1	0	3	0	3

* No individual litter data available

Control data of studies 1-11 were included in the study report