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Review

Safety evaluation of neem (*Azadirachta indica*) derived pesticides

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Abstract

The neem tree, *Azadirachta indica*, provides many useful compounds that are used as pesticides and could be applied to protect stored seeds against insects. However in addition to possible beneficial health effects, such as blood sugar lowering properties, anti-parasitic, anti-inflammatory, anti-ulcer and hepatoprotective effects, also toxic effects are described. In this study we present a review of the toxicological data from human and animal studies with oral administration of different neem-based preparations. The non-aqueous extracts appear to be the most toxic neem-based products, with an estimated safe dose (ESD) of 0.002 and 12.5 μg/kg bw/day. Less toxic are the unprocessed materials seed oil and the aqueous extracts (ESD 0.26 and 0.3 mg/kg bw/day, 2 μl/kg bw/day respectively). Most of the pure compounds show a relatively low toxicity (ESD azadirachtin 15 mg/kg bw/day). For all preparations, reversible effect on reproduction of both male and female mammals seem to be the most important toxic effects upon sub-acute or chronic exposure. From the available data, safety assessments for the various neem-derived preparations were made and the outcomes are compared to the ingestion of residues on food treated with neem preparations as insecticides. This leads to the conclusion that, if applied with care, use of neem derived pesticides as an insecticide should not be discouraged.

Keywords: Toxicity; Health evaluation; Oral administration; NOAEL; Neem-derived pesticides

1. Introduction

In the search for environmentally safe pesticides, much research has been done on the use of plants for the protection of crops in the field or in storage. Especially in tropical regions, the application of botanical material to protect a crop against insects is often traditional and centuries old.

The one plant species that is probably best investigated for its effects against insects is the neem tree, *Azadirachta indica* A. Juss. (Meliaceae). All parts of this tropical tree contain bitter compounds (Van der Nat et al., 1991; Chawla et al., 1995) that often have an antifeedant effect and can interfere with hormonal processes in insects (Schmutterer, 1990; Ascher, 1993). Extracts or crude parts of this plant are often mixed with seeds such as maize, grain, rice and beans in storage to protect these seeds against insects. Results of storage tests mostly indicate that the leaf powder, the seed oil and all kinds of extracts do indeed have a negative effect on the seed-eating insects.

However, if products of this tree are to be used to treat stored seeds against insects, the mammalian consumers of these seeds ought not to be affected by residues of this treatment. Much controversy exists about the use of especially the seed oil of the neem tree. On the one hand, it is claimed that the oil is easily removed from seeds and leaves no negative effect on the taste of the seeds (Anonymous, 1995). On the other hand, Naik and Dumbre (1985) mentioned that it is hardly possible to remove the very bitter oil from treated seeds, and that the germination of treated seeds is negatively

Abbreviations: ADI, acceptable daily intake; bw, body weight; CNS, central nervous system; DMBA, 7,12-dimethylbenz[α]anthracene (carcinogenic agent); ED_{50}, medium effective dose; ESD, estimated safe dose; ESR, erythrocyte sedimentation rate; GGT, gamma glutamyl transpeptidase; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione-S-transferase; Hb, haemoglobin; LC_{50}, medium lethal concentration; LD_{50}, medium lethal dose; L(O)AEL, lowest (observed) adverse effect level; N(O)AEL, no (observed) adverse effect level; P450, cytochrome P450; PCV, packed cell volume [haematocrit]; SGOT, aspartate aminotransferase [glutamic oxaloacetate transaminase]; SGPT, alanine aminotransferase [glutamic pyruvate transaminase]; TEC, total erythrocyte count; TLC, total leukocyte count

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influenced. Moreover, the oil can turn rancid (De Groot, 1991) and is easily contaminated with aflatoxins (Sinniah et al., 1982).

Given the use of various neem-derived products as pesticides and the realistic chances of residues derived from the treatments still being present at the time of consumption, there may be a risk for consumers. Therefore, in this study we present a review of the toxicological data from human and animal studies with oral administration of different neem-based preparations. These preparations can consist of crude plant parts, the seed oil, aqueous extracts of parts of the tree, extracts obtained with non-aqueous solvents, the pure bioactive insecticide ingredients and commercially available neem-based pesticides.

The first three application modes would be best applicable for low-resource farmers in tropical countries, where no complex extractions can be performed due to a lack of solvents and equipment. The other application modes could be valuable in countries where rules are strict and where law requires exact definition of the ingredients of pesticides. We do not discuss preparations of seed cake, the rest product after oil extraction, since the active ingredients are mostly removed from this material and the effects on mammals are usually little pronounced (Gangopadhyay et al., 1979; Nath et al., 1989; Ramu et al., 1997). In the following sections, the toxicity data and a risk assessment on the following neem-based materials are presented: unprocessed material, seed oil, aqueous extracts, non-aqueous extracts, pure compounds and commercial products.

2. Unprocessed material

Many parts of the neem tree are used in an unprocessed form. For such an application, raw plant materials, mainly leaves and fruit kernels are harvested and applied immediately or after drying or grinding. The toxic components in these materials are not concentrated and the toxicity is expected to be less pronounced than in extracts. In areas where the tree grows, accidental ingestion of these materials by grazing cattle or playing children is likely, since the tree is rather common.

2.1. Effects on humans

Table 1 summarises the reported effects of unprocessed neem materials on humans. Alam et al. (1990) reported the use of neem leaves against diabetes mellitus in indigenous medicine in India of which the results are highly satisfactory to the local population. A disadvantage of such self-medication is reported by Kadiri et al. (1999) who found that traditional neem leaf-based medicines, taken to treat febrile illness, abdominal upset or to induce abortion or infertility had acute toxic effects. The major features observed were oliguria or anuria, jaundice and anaemia. The picture was consistent with acute tubular necrosis in all the cases and the mechanisms causing the effects were intravascular haemolysis, hepatotoxicity and direct nephrotoxicity. Three out of 53 patients died. Another disadvantageous feature was found in the allergenicity of the neem pollen. Chakraborty et al. (1998), in a survey of the aeropalynoflora in India, identified 46 pollen types. The abundance of pollen of the neem tree was relatively low, but when subjected to clinical investigation to determine their degree of allergenicity on adult respiratory allergic patients, they appeared to be highly allergenic.

Since none of these reports mentioned any exposure data, they cannot be used to assess the risks associated with the exposure to unprocessed neem materials.

2.2. Effects on animals

The effects of unprocessed neem materials on animals are summarised in Table 2.

2.2.1. Acute effects

Acute toxicity of unprocessed material in animals was reported only for a sheep that ate neem leaves. Ingestion resulted in nervous symptoms (head movements, walking in circles) with dyspnoea, an increase in body temperature, hepatic failure and tympanites. The symptoms lasted for 12 h and were followed by the death of the animal (Ali and Salih, 1982).

More positively, administration of leaf sap caused an anti-anxiety effect in rats at low doses, while high doses did not cause such an effect (Jaiswal et al., 1994). In sheep, kernel powder caused a decrease in the number of nematode eggs in their faeces, and an increase in body weight (Ahmed et al., 1994).

2.2.2. Subacute effects

An important effect upon subacute exposure to leaf powder is that on reproductive ability in male rats. Leaf powder caused a decrease in the weight of the seminal vesicle and

Table 1
Effects of unprocessed neem materials on humans

Plant part	Administration	Dose	Duration	Observed effect(s)	Reference
Leaves	Oral	Two tablets	[a]	Anti-diabetic effect	Alam et al. (1990)
Pollen	Skin prick test	[a]	Once	Allergenic effect	Chakraborty et al. (1998)
Leaves	Oral, intravaginally	[a]	[a]	Acute renal failure	Kadiri et al. (1999)

[a] Not specified in reference.

Table 2
Effects of unprocessed neem materials on animals

Plant part	Test animal	Dose	Duration (days)	Observed effect(s)	Reference
Acute					
Leaf sap	Rats	10–800 mg/kg bw	1	Anti-anxiety effect	Jaiswal et al. (1994)
Kernel powder	Sheep	75, 100 mg/kg bw	1	Effect against intestinal nematodes	Ahmed et al. (1994)
Leaves	Sheep	100 g/sheep	1	Acute toxicity	Ali and Salih (1982)
Subacute					
Leaf powder	Rats	20; 40; 60 mg/rat	24	Reduced sperm count and mobility, sperm malformations	Parveen et al. (1993)
Leaf powder	Rats	20, 40, 60 mg/rat	24	Reduced epididymal function	Kasturi et al. (1995)
Leaf powder	Rats	20, 40, 60 mg/rat	24	Reduced weigth seminal vesicles and ventral prostate	Kasturi et al. (1997)
Leaf powder	Rats	100 mg/rat	24	Changes in testes	Joshi et al. (1996)
Semi-chronic					
Leaf powder	Rats	100 mg/rat	48	Sperm parameters	Aladakatti et al. (2001)
Fruits	Rabbits	2000 mg/rabbit/day	70	Control of hydatidosis	Tanveer et al. (1998)
Leaves	Cattle	10 (=2 g/rat), 20, 30% in diet	98	Effect against intestinal nematodes	Pietrosemoli et al. (1999)

the ventral prostate (Kasturi et al., 1997), a reduction in the sperm count and sperm motility as well as an increased percentage of malformed sperm (Parveen et al., 1993). Moreover, at a dose of 100 mg/rat, a reduction in the diameters of the seminiferous tubule was observed. Gradual recovery in histological and biochemical parameters was found after termination of the treatment (Joshi et al., 1996). At slightly lower doses, the height of the epithelium in caput and cauda epididymis was reduced dose-dependently. The lumen of the caput was packed with lymphocytes and the serum testosterone concentration was decreased (Kasturi et al., 1995). Biochemically, the leaf powder caused decreases in protein content and acid phosphatase activity, and increases in activities of alkaline phosphatase and lactate dehydrogenase (Kasturi et al., 1997), and in total free sugar, glycogen and cholesterol contents (Joshi et al., 1996).

2.2.3. Semi-chronic effects

In a semi-chronic study, Aladakatti et al. (2001) found that leaf powder in rats caused a decrease in total sperm-count and in sperm motility. The relative percentage of abnormal sperm increased. Since the effects of the powder were annihilated when testosterone was administered simultaneously, the authors suggested that the effects were due to an androgen deficiency, thereby affecting the physiological maturation of sperm.

A positive effect against intestinal nematodes was found for cattle upon neem leaf feeding without any effect on the weight gain (Pietrosemoli et al., 1999). In rabbits, neem fruits caused decreased serum activities of acid phosphatase, alkaline phosphatase and glucose and an improvement of glutamic oxaloacetate transaminase (SGOT), glutamic pyruvate transaminase (SGPT), cholesterol, total protein and bilirubin values (Tanveer et al., 1998).

2.3. Evaluation of the data

As the human data are derived from case studies and no exposure levels were presented these data cannot be used as a starting point for risk assessment of the unprocessed material. For that reason hazard characterisation was only based on animal data. All together, the major toxic outcome of unprocessed neem materials in animals may be the effects on male fertility upon sub-acute or semi-chronic exposure to the leaf powder at 20–100 mg/rat.

For the effects on protein content of the seminal vesicle (Kasturi et al., 1997) a no adverse effect level (NAEL) of 6.6 mg/rat (26.4 mg/kg body weight (bw)) could be derived from the reported dose-effect data using log-linear extrapolation. When this effect on protein content of the seminal vesicle is considered to be the most sensitive toxic parameter, the NAEL thus derived can be used for a safety assessment for human consumption. Using the standard safety factors of 10 for inter- and 10 for intra-species extrapolations, this results in a calculated acceptable daily intake (ADI) for human consumption of $26.4 \times 0.1 \times 0.1 = 0.264$ mg/kg bw/day. For a 70 kg weighing adult, this amounts to 70×0.264 mg/kg $=$ 18.5 mg unprocessed leaves/day.

This value gives an indication of the range in which a safe dose for daily human exposure to unprocessed neem material i.e. unprocessed leaves could be found.

3. Seed oil

The seeds of the neem tree are rich in oil, which can be extracted to produce seed oil. In many tropical countries, this oil is sold as a household medicament to use against all kinds of inconveniences and diseases such as muscle-aches, malaria, tuberculosis and even diabetes.

3.1. Effects on humans

In Table 3, the reported effects of neem oil on humans are summarised. Two cases were described where oral administration to young children resulted in acute toxic effects. The oil, even in small amounts was reported to cause

Table 3
Effects of neem oil on humans

Administration	Dose	Duration	Observed effect(s)	Reference
Oral	'Droplets' and 5 ml	Once	Encephalopatic effect	Lai et al. (1990)
Oral	12 ml	2 days	Acute toxicity	Sinniah et al. (1982)

toxic encephalopathy. Features were vomiting, drowsiness, tachypnoea, and recurrent generalised seizures. Laboratory tests showed that the oil causes leukocytosis and metabolic acidosis (Lai et al., 1990). Sinniah et al. (1982) reported the case of a child, who died after administration of the oil as treatment for a cough. Autopsy findings revealed changes in the liver and kidneys consistent with Reye's syndrome but unlike those described in acute aflatoxicosis. As aflatoxins have been identified in oil samples, the toxic action of the oil may have been due to the synergistic effects of aflatoxins and other toxic components in the oil.

In indigenous medicine in India, the oil is considered to have a contraceptive activity (Lakshmanan and Naryanan, 1990). Other studies in India revealed that the oil is effective as a mosquito-repelling agent, and thus helps to prevent malaria. The oil, either applied topically (Sharma et al., 1993; Kant and Bhatt, 1994; Mishra et al., 1995) or burnt in a lamp (Sharma and Ansari, 1994) provided protection from Anopheles and Culex mosquitoes.

The doses reported in the table (5 and 12 ml per young child) are clearly toxic, which implies that safety values for human consumption calculated from animal studies should be much below ±0.20 ml/kg bw (taking 25 kg for the body weight of a child).

3.2. Effects on animals

The effects of oral administration of oil to animals are summarised in Table 4.

3.2.1. Acute effects

Gandhi et al. (1988) reported acute toxicity after ingestion of the oil by rats and rabbits. The oil-induced dose- and time-dependent effects on motor activity, respiration and on the orientation within the cage and the animals had diarrhoea, tremors and convulsions. The medium lethal dose (LD_{50} value) was 14.1 ml/kg bw for rats and, showing similar symptoms, 24.0 ml/kg bw for rabbits. The oil was not toxic to mice at lower doses, but at high dose, treated animals showed hyper-excitability to sound and touch, convulsive jerks, laboured respiration, and some animals died (Tandan et al., 1995).

In rats, administration of neem oil during the first few days of pregnancy had a higher abortive effect than later administration. At a dose of 6 ml/kg bw, even 3 out of 13 adult animals died (Lal et al., 1987). Administration of oil increased tail flick reaction time and reduced induced writhing (Khosla et al., 2000a). In normal and hyperglycaemic rats, administration of oil caused a lowering of the blood glucose (Dixit et al., 1986).

3.2.2. Subacute effects

Due to subacute administration (2.0–4.6 ml/kg bw), the oestrous cycle of female rats was disturbed resulting in a reduction in fertility. The body weight was reduced when the animals were administered a high dose of neem oil (Dhaliwal et al., 1998). Subacute administration of seed oil led to lowered blood sugar levels in normal and diabetic rabbits (Khosla et al., 2000b).

3.2.3. Semi-chronic effects

Chronic effects are described by Lakshminarayana (1987) who investigated the neem tree as a potential source of edible oil, and by Rukmini (1987) who concluded from a study in rats that such oil derived from the neem tree would be safe. Debitterised neem oil was found useful as animal feed (Rukmini et al., 1991). A three-generation study in male and female rats fed a diet containing debitterised oil did not show

Table 4
Effects of neem oil on animals

Test animal	Dose	Duration (days)	Observed effect(s)	Reference
Acute				
Mice	1.0–28.2 g/kg bw	1	Toxicity	Tandan et al. (1995)
Rats	2 ml/kg bw	1	Anti-nociceptive effect	Khosla et al. (2000a)
Rats	4, 6 ml/kg bw	2–3	Abortive in females	Lal et al. (1987)
Rabbits, rats	10–80 ml/kg bw	1	Acute toxicity	Gandhi et al. (1988)
Rats	200 mg/rat	1	Anti-diabetic effect	Dixit et al. (1986)
Subacute				
Rats	2.0, 3.3, 4.6 ml/kg bw	18	Anti-fertility in females	Dhaliwal et al. (1998)
Rabbits	5 ml/kg bw	28	Anti-diabetic effect	Khosla et al. (2000b)
Semi-chronic				
Rats	10% in diet	3 generations (130 each)	No toxicity, no effect on reproduction	Chinnasamy et al. (1993)

any adverse effects on the general health or reproductive parameters. The mean organ weights and the histopathological evaluation of all the organs were similar. The extract was negative in the Ames test (Chinnasamy et al., 1993).

3.3. Evaluation of the data

All together neem oil shows acute toxicity at doses of 14 and 24 ml/kg bw for rats and rabbits respectively. Upon use as an insecticide, it is unlikely that these high levels of intake will be encountered when considering human intake of residues on treated beans, since these doses in rats and rabbits would amount to about 1000 ml of oil or more for a 70 kg adult. The most relevant adverse effect reported in neem-oil exposed animals seems to be the anti-fertility effect in female rats observed upon sub-acute exposure to 2.0–4.6 ml/kg bw (Dhaliwal et al., 1998). This implies a lowest observed adverse effect level (LOAEL) for the antifertility effect in female rats of 2.0 ml/kg bw. Taking a standard safety factor of 10 for extrapolation of the LOAEL to a no adverse effect level (NAEL) this results in a value of 0.2 ml/kg bw. Extrapolating this to the human situation using the standard safety factors for intra- and interspecies extrapolations this results in an ADI of $0.2 \times 0.1 \times 0.1$ ml/kg bw amounting to 0.002 ml/kg bw. This implies that a daily intake of 0.14 ml oil for an adult of 70 kg can be considered safe. This value of 0.002 ml/kg bw is 100 times lower than the dose of 0.2 ml/kg bw reported to be toxic in children.

4. Aqueous extracts

A simple way to prepare a plant extract is the soaking of plant material in water. This provides aqueous neem extracts.

4.1. Effects on humans

Kroes et al. (1993) reported that in Sri Lankan medicine a fermented decoction of neem bark is taken as a drug with immunomodulatory activity. An in vitro haemolytic assay proved that the human complement system and the activity of polymorphonuclear leukocytes from healthy volunteers were inhibited.

4.2. Effects on animals

As shown in Table 5, the investigations on effects of aqueous neem extracts on animals are numerous, and reveal in most cases beneficial rather than harmful effects.

4.2.1. Acute effects

Leaf extract caused a moderate decrease of the blood glucose levels in mice (Mossa, 1985). It produced hypoglycaemia in normal rats. The clotting time of blood was higher than normal. Serum cholesterol level increased with a concomitant decrease in liver fat and a dose-related drop in liver proteins (El Hawary and Kholief, 1990). The extract had toxic effects, as reflected by body weight loss and high percentage mortality.

Leaf extract was effective against *Plasmodium yoelii nigeriensis* in mice (Obaseki and Jegede Fadunsin, 1986).

The tail flick reaction time increased and a reduction in induced writhing was observed in rats that were administered leaf extract. Naloxone pre-treatment partially reversed the effects. The effects of the leaf extract were more pronounced than those of the seed oil (Khosla et al., 2000a). Leaf extract reduced gastric ulcer severity in rats and decreased gastric mucosal damage (Garg et al., 1993a). Chemically-induced carcinogenesis with accompanying high levels of lipid peroxidation and low levels of glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and gamma glutamyl transpeptidase (GGT) in rats could be effectively reduced with leaf extract. A five-day pre-treatment with leaf extract decreased the formation of lipid peroxides and enhanced the levels of antioxidants and detoxifying enzymes in the stomach, the liver and circulation (Arivazhagan et al., 2000a).

4.2.2. Subacute effects

Dose-dependent subacute effects were observed in mice, where aqueous leaf extract reduced tri-iodothyronine (T3) and increased serum thyroxine (T4) concentrations and hepatic lipid peroxidation and decreased glucose-6-phosphatase activity while enhancing the activities of superoxide dismutase and catalase (Panda and Kar, 2000). Treatment of mice had no influence on liver, spleen, thymus or body weight indices but it caused elevated IgM and IgG concentrations and anti-ovalbumin antibody titres and an enhancement of macrophage migration inhibition and footpad thickness (Ray et al., 1996). Livers of paracetamol-induced rats were normal in appearance and histology after leaf extract administration. The extract caused a reduction of paracetamol-induced high serum levels of SGOT, SGPT and GGT (Bhanwra et al., 2000). Subchronic administration of leaf extract caused a decrease in blood sugar levels in normal and diabetic rabbits. The extract was more effective than seed oil (Khosla et al., 2000b).

4.2.3. Semi-chronic effects

Rats treated with leaf extract showed decreased appetite, body weight and pupillary reflex. Their total erythrocyte count (TEC) and blood glucose level were reduced. Histopathological studies revealed congestion in the liver, kidneys, lungs and brain (Hore et al., 1999). The body weight of goats and guinea pigs decreased due to administration of leaves to their drinking water. Both acute and chronic toxicity were evident through signs of weakness, loss of condition and depression. Decreases in heart, pulse and respiratory rates were observed and diarrhoea, tremors and ataxia occurred in some animals. TEC, packed cell volume (PCV) and haemoglobin (Hb) decreased slightly, whereas the activities of SGOT, sorbitol dehydrogenase

Table 5
Effect of aqueous neem extract on animals

Plant part	Test animal	Dose (equivalent weight of leaves fresh (f) or dry (d))	Duration (days)	Observed effect(s)	Reference
Acute					
Leaves	Rats	10–160 mg/kg bw (10–160 d)	1	Gastric anti-ulcer effect	Garg et al. (1993a)
Leaves	Mice	0.5 ml/mouse (12.5 mg d)	1	Anti-diabetic effect	Mossa (1985)
Leaf extract	Rats	100 mg/kg bw[a]	5	Stimulation of GGT activity	Koner et al. (1997)
Leaves	Mice	100–400 mg/kg bw (690–2778 d)	1	Effective against malaria parasites	Obaseki and Jegede Fadunsin (1986)
Leaves	Mice	125–500 mg/kg bw (166–666.7 f)	4	Slight effect against malaria parasites	Abatan and Makinde (1986)
Leaves	Rats	200, 300 mg/kg bw (200, 300 d)	7	Anti-diabetic effect and toxicity	El Hawary and Kholief (1990)
Leaves	Rats	250 mg/kg bw (250 f)	5	Reduced lipid peroxidation, increased antioxidant status	Arivazhagan et al. (2000a)
Leaves	Rats	500 mg/kg bw[a] f)	1	Anti-nociceptive effect	Khosla et al. (2000a)
Subacute					
Leaves	Mice	10, 30, 100 mg/kg bw[a]	21	Modulation of immune responses	Ray et al. (1996)
Leaves	Mice	40, 100 mg/kg bw (200, 500 d)	20	Adverse effect on thyroid function	Panda and Kar (2000)
Leaves	Rats	500 mg/kg bw[a] d)	9	Hepatoprotective effect	Bhanwra et al. (2000)
Leaves	Rabbits	500 mg/kg bw[a] d)	28	Anti-diabetic effect	Khosla et al. (2000b)
Semi-chronic					
Leaves suspension	Goats, guinea pigs	50–2000 mg/kg bw (50–2000 f and d)	5, 56	Toxicity	Ali (1987)
Leaves	Hamsters	100 mg/kg bw[a]	98	Anti-carcinogenic effect	Balasenthil et al. (1999a)
Leaves	Hamsters	100 mg/kg bw[a]	98	Anti-carcinogenic effect	Balasenthil et al. (1999b)
Leaves	Rats	100 mg/kg bw[a]	49	Toxicity	Hore et al. (1999)
Leaves	Rats	100 mg/kg bw (100 f)	182	Chemopreventive potential	Arivazhagan et al. (1999a)
Leaves	Rats	100 mg/kg bw (100 f)	182	Effects on circulating lipid peroxides and antioxidants	Arivazhagan et al. (1999b)
Leaves	Rats	250 mg/kg bw (250 f)	182	Lipid peroxidation and antioxidant status	Arivazhagan et al. (2000b)
Twigs, fruits	Rats	0.1–1.6% in diet (0.25–4 g d)	70	Effect on blood constituents	Parshad et al. (1994)
Leaves	Rats	1000 mg/kg bw[a]	42	Anti-diabetic effect	Bajaj and Srinivasan (1999)
Kernels	Mice	[a]	30	Anti-tubercular effect	Usha and Saroja (2001)

[a] Not specified in reference.

and the concentrations of cholesterol, urea, creatine and potassium increased. Liver and kidneys were most affected (Ali, 1987). However, treatment of rats with leaf extract resulted in decreases in total testosterone, total bilirubin and potassium in serum. There were increases in PCV, mean corpuscular Hb concentration, red blood cell, white blood cell and lymphocyte counts, but no cytotoxic effects were observed (Parshad et al., 1994).

The aqueous leaf extract showed a fall in blood glucose levels in diabetic rats (Bajaj and Srinivasan, 1999). Administration of kernel extract to mice protected their tissues from the damage caused by *Mycobacterium tuberculosis* (Usha and Saroja, 2001).

4.3. Evaluation of the data

The effects of aqueous extracts are ambiguous. Many of the studies do not report dose-effect relations. Mostly positive effects are mentioned, even after administration of high doses, but toxic effects were observed at concentrations of 200 mg/kg bw resulting in death of treated goats (Ali, 1987). Effects on reproduction were only indirectly mentioned as a decrease in testosterone (Parshad et al., 1994).

The most relevant NOAEL is 30 mg/kg bw/day at which there is no modulation of the immune responses (Ray et al., 1996). Based on this NOAEL and using the standard safety factors, an ADI of $30 \times 0.1 \times 0.1 = 0.3$ mg/kg bw/day can be derived as the safety level of aqueous neem extracts.

5. Non-aqueous extracts

If non-aqueous solvents are available, these can be used to extract more apolar, possibly more active constituents from neem material. These extracts, containing the active compounds and not the ineffective bulk of plant material, are often more active than the crude materials they were obtained from. As in most cases analytical characterisation of the extracts is missing, it is impossible to compare the toxicity of the various extracts to those of possible constituents like azadirachtin.

5.1. Effects on humans

In Table 6, the effects of several non-aqueous neem extracts on humans are summarised. Via ammonium precipitation, it was possible to isolate active allergenic components from neem pollen. A skin prick test on human volunteers revealed several major allergens in neem pollen extract (Karmakar and Chatterjee, 1994).

A pessary including neem leaf extract did not show any toxicity and there were no side-effects whereas it was effective in prevention of pregnancy in most of the volunteers (Talwar et al., 1997). Bombarde and Bombarde (1994) reported the traditional use of neem as a drug to treat patients with diabetes.

5.2. Effects on animals

Effects of orally administered non-aqueous extracts on animals are summarised in Table 7.

5.2.1. Acute effects

Extracts were more repellent than the powders (Oguge et al., 1997). Symptoms of acute toxicity of an *acetone* leaf extract were a decrease in spontaneous activity, respiratory rate and body and limb tone in mice. Decreased responses to the environment, piloerection and a dose-dependent hypothermia were observed as well (Singh et al., 1987). Two fractions of an acetone leaf extract showed central nervous system (CNS) depressant activity in mice as evidenced by a reduction in locomotor activity. Both fractions caused reductions in blood pressure and heart rate in rats without showing diuretic activity (Singh et al., 1990).

Methanol extracts of bark and leaves had a pronounced anti-inflammatory and a good antipyretic effect in rats and rabbits. Methanol bark extract established an anti-thrombotic effect in mice (Olajide, 1999). A test for acute oral toxicity in mice showed an LD_{50} value of approximately 13 g/kg bw (Okpanyi and Ezeukwu, 1981).

The ether soluble fraction of *alcohol* leaf extract showed good analgesic activity in acute inflammatory pain in rats and mice and it did not show acute toxicity in mice (Tandan et al., 1990).

Petrol ethanol leaf extract did exhibit anti-inflammatory activity in rats and had an analgesic effect in mice. Antipyretic activity required administration at high dosage. Acute toxicity symptoms in mice were effects on the motor activity, on orientation, a reduced reaction to pain and convulsions. The oral LD_{50} of the extract was 22 g/kg bw (Koley et al., 1994).

Ethanol leaf extract had an anti-inflammatory activity through inhibition of the proliferative phase of inflammation (Chattopadhyay, 1998). Ethanol leaf extract dose-dependently induced mitotic chromosome abnormalities in bone marrow cells of mice. Gross type abnormalities

Table 6
Effects of neem extracts made with non-aqueous solvents on humans

Plant part	Administration	Dose	Duration	Obserbed effect(s)	Reference
Pollen	Skin prick test	[a]	Once	Allergenic effect	Karmakar and Chatterjee (1994)
Leaf	Intravaginally	[a]	7 days	Prevention of pregnancy	Talwar et al. (1997)

[a] Not specified in reference.

Table 7
Effects of neem extracts made with non-aqueous solvents on animals

Plant part	Solvent	Test animal	Dose (equivalent weight of leaves fresh (f) or dry (d))	Duration (days)	Observed effect(s)	Reference
Acute						
Leaves	Alcohol	Mice, rats	[a]	1	Effect on blood constituents	Chattopadhyay et al. (1993b)
Leaves	Acetone	Mice	50, 100, 200 mg/kg bw[a] (d)	1	Neuro-psychophar-macological effect	Singh et al. (1987)
Leaves	Alcohol	Mice, rats	50–400, 0.25–8.0 mg/kg bw (996–7968.5–159 d)	1	Anti-diabetic effect	Chattopadhyay (1999)
Leaves	Acetone	Mice, rats	100 mg/kg bw[a] (d)	1	Effect on central, autonomic and cardiovascular systems	Singh et al. (1990)
Leaves	Ethanol	Rats	100, 200 mg/kg bw (1992, 3984 d)	1, 7	Anti-inflammatory action	Chattopadhyay (1998)
Leaves	Ethanol	Rabbits	200 mg/kg bw (3984 d)	1, 7	Anti-diabetic effect	Chattopadhyay (1996)
Leaves	Ethanol	Rats	500 mg/kg bw[a]	1	Reduction in hepatic glycogen	Chattopadhyay et al. (1993a)
Leaves	Ethanol	Mice	500, 1000, 2000 mg/kg bw[a] (d)	7	Genotoxicity	Awasthy et al. (1995)
Leaves	Ethanol	Mice	500, 1000, 2000 mg/kg bw[a] (d)	7	Genotoxicity	Awasthy et al. (1999)
Leaves	Alcohol	Rats	1000 mg/kg bw[a]	7	Hepatoprotective effect	Chattopadhyay et al. (1992)
Leaves	Petrol ethanol	Mice, rats	0.1–1.0, 10–40 g/kg bw (3.7–37.3, 373–1493 f)	1	Anti-inflammatory effect, toxicity	Koley et al. (1994)
Leaves	Ethanol	Mice, rats	0.1–1.0, 1.3, 10 g/kg bw (15.9–158.7, 206, 1587.3 d)	1	Analgesic effect, no acute toxicity	Tandan et al. (1990)
Leaves, seeds	[a]	Baboons rats	6 ml and 0.6 ml/animal[a]	3, 6	Effect on reproduction	Talwar et al. (1997)
Seeds	[a]	Rodents	0.4 ml/animal[a]	3	Resorption of embryo's	Mukherjee et al. (1996a)
Seeds	Hexane	Rats	25, 50, 75, 100%[a]	3	Abrogation of pregnancy	Mukherjee et al. (1999)
Fruits, leaves	Methanol	Rodents	Soaked food[a] (d)	5	Anti-feedant effect, toxicity	Oguge et al. (1997)
Bark, leaves	Methanol	Mice, rabbits, rats	0.4–12.8 g/kg bw (32.9–1052 f)	1	Anti-inflammatory and anti-pyretic effects, toxicity	Okpanyi and Ezeukwu (1981)
Bark	Methanol	Mice	100 mg/kg bw[a] (d)	1	Anti-thrombotic effect	Olajide (1999)
[a]	[a]	Mice	2 ml/mouse[a] (f)	1	No effect on malaria parasites	Bray et al. (1990)
Subacute						
Leaves	Ethanol	Rats	100 mg/kg bw[a]	21	Effect on reproduction	Choudhary et al. (1990)
Leaves	Ethanol	Rats	100 mg/kg bw (1000 f)	28	Hypolipidemic effect	Chattopadhyay (1995)
Leaves	Chloroform hexane, methanol	Rats	12.50% in diet (12.5% f)	14	Anti-mutagenic, anti-carcinogenic effect	Kusamran et al. (1998b)
Flowers	Chloroform hexane, methanol	Rats	12.50% in diet (12.5% f)	14	Effect on hepatic enzyme activities	Kusamran et al. (1998a)
Semi-chronic						
Leaves	Alcohol	Mice	500, 1000, 2000 mg/kg bw[a]	42	Genotoxicity Sperm deformations	Awasthy (2001)
Bark, flowers, oil	[a]	Rats	[a]	[a]	Reduced male reproduction	Dixit et al. (1992)
Flowers	Ethanol	Rabbits	500 mg/kg bw[a] (d)	30, 60	Hypolipidaemic effects	Purohit and Daradka (1999)
Husks, seeds	Petroleum ether	Rats	566, 360 mg/kg bw[a] (f)	60	Effect on blood constituents	Gupta et al. (2001)
Husks, kernels	Petroleum ether	Rats	1000 mg/kg, 72 mg/kg bw[a]	60	Toxicity	Kataria et al. (2000)
[a]	[a]	Rats	[a]	[a]	Reduced ovarian activity	Mishra (1996)

[a] Not specified in reference.

appeared even at the lowest dose and remained unchanged in frequency at higher doses. The extract caused increased incidence of structural changes of metaphase chromosomes. A constituent of the extract probably interfered with DNA to yield chromosome strand breakage or produced spindle disturbances, inducing genotoxic effects (Awasthy et al., 1999).

Ethanol leaf extract in itself had no effect on peripheral utilisation of glucose (Chattopadhyay, 1996). At doses higher than 50 mg/kg bw the extract decreased the blood sugar level. The LD_{50} value in mice was 4.6 g/kg bw (Chattopadhyay, 1999). Ethanol leaf extract did not alter the hepatic glycogen content in normal rats, but in glucose fed rats or in combination with insulin it reduced the hepatic glycogen content (Chattopadhyay et al., 1993a).

Examination in rodents previously treated with seed extracts revealed complete resorption of embryos on day 15 of pregnancy (Mukherjee et al., 1996a).

Hexane seed extract, in contrast to ethanol and water extracts, completely abrogated pregnancy. Restoration of fertility was observed in subsequent cycles and no further toxic effects were found (Mukherjee et al., 1999). Non-aqueous leave extracts did not show anti-malarial activity in vitro or in vivo in mice (Bray et al., 1990).

5.2.2. Subacute effects

Neem flowers in the diet of rats increased hepatic GST activity and reduced the activities of cytochrome P450 (P450), aniline hydroxylase and aminopyrine-N-demethylase. The flowers contain phase II enzyme inducers and compounds capable of repressing monooxygenases, especially those involved in metabolic activation of chemical carcinogens (Kusamran et al., 1998a). Leaf extracts contain a weak antimutagen, as was revealed in an Ames' test. The mechanism of the anti-mutagenicity may be through inhibition of the activity of metabolic-activating enzymes in the liver (Kusamran et al., 1998b). Treatment of rats with ethanol leaf extract reduced elevated serum levels of cholesterol, total lipids and triglycerides (Chattopadhyay, 1995). Ethanol leaf extract did not interfere with spermatogenesis (Choudhary et al., 1990).

5.2.3. Semi-chronic effects

No changes in Hb, PCV, total leukocyte count (TLC), mean corpuscular Hb concentration and the blood glucose level were found due to treatments, but blood SGOT and SGPT decreased. Serum protein, serum cholesterol, plasma total lipids and GST increased, while plasma phospholipids and erythrocyte acetylcholinesterase decreased (Gupta et al., 2001). Neem extract was active against some fungi, bacteria, and viruses. In another study, cholesterol and triglycerides levels in blood serum and liver decreased in rats (Sharma et al., 1999).

In a literature overview Kumar and Jattan (1995) reported the contraceptive activity of extracts in male and female rats and mice after different kinds of administration. In rats, extract reduced the weight of ovaries and uterus. Contents of ascorbic acid and cholesterol of ovaries increased (Mishra, 1996). Extracts of bark, flowers and seed oil induced reversible infertility in male rats, like decreases in spermatid number. Neem extracts reduced blood glucose levels (Dixit et al., 1992). The alcohol extract of leaves reduced the sperm count and increased the frequency of spermatozoa with abnormal head morphology (Awasthy, 2001).

5.3. Evaluation of the data

The major negative effects of non-aqueous neem extracts are effects on male and female reproductive ability.

Singh et al. (1987) found a LOAEL for neuro-psychopharmacologic effects of 12.5 mg acetone leaf extract/kg bw in mice upon acute exposure. Using an extra standard safety factor 10 for extrapolation of the LOAEL to a NAEL this implies that a calculated safe dose for acute human exposure should be lower than $12.5 \times 0.1 \times 0.1 \times 0.1 = 0.0125$ mg acetone extract/kg bw.

6. Pure compounds

For the use as pesticides in the developed countries, for legislation purposes, the exact ingredients of neem should be known. The active compounds and their concentration differ with the plant parts used and with their age, growing conditions and climate (Schoonhoven et al., 1998). Many of the secondary compounds of the neem have been identified (Van der Nat et al., 1991), purified and some have been tested for their effects on mammals. These include for example azadirachtin, nimbolide and nimbinin.

6.1. Effects on humans

Effects of pure neem derived compounds on human health are not documented. However, Beard (1989) mentioned that azadirachtin was not toxic to humans.

6.2. Effects on animals

In Table 8, documentation of effects of pure neem derived compounds on animals is summarised.

6.2.1. Acute effects

When rats were treated with azadirachtin, increased serum SGOT and SGPT activities and bilirubin content were observed. Histopathological studies showed pathological changes in the liver in terms of congestion, hydropic degeneration, necrosis and lymphocytic infiltration (Abdel Megeed et al., 2001).

Upon acute exposure, nimbidin, isolated from seeds, dose-dependently reduced acute paw oedema in rats, and suppressed induced arthritis and fluid exudation in induced granuloma. The medium effective dose (ED_{50}

Table 8
Effect of pure compounds of the neem tree on animals

Compound	Test animal	Dose	Duration (days)	Observed effect(s)	Reference
Acute					
Azadirachtin	Rats	0.1 LD_{50} = 57 ppm	1–3	Effect on liver function	Abdel Megeed et al. (2001)
Limonoids	Mice	2 ml/mouse	1	Effect against intestinal parasites	Bray et al. (1990)
Limonoids	Mice	135 mg/kg bw	4	No anti-malarial effect	Bray et al. (1985)
Nimbidin	Rats	20, 30, 40 mg/kg bw	1	Anti-arthritic and anti-inflammatory effect	Pillai and Santhakumari (1981)
Subacute					
Azadirachtin	Rats	0.1 LD_{50}	21	Effect on blood constituents	Radwan et al. (2001a)
Azadirachtin	Rats	500, 1000, 1500 mg/kg bw	21	No fetotoxicity or teratogenicity	Srivastava and Raizada (2001)
Nimbidin	Dogs, guinea pigs, rats,	20–80 mg/kg bw	1, 10, 28	Anti-ulcer effect	Pillai and Santhakumari (1984a)
Semi-chronic					
Azadirachtin	Rats	0.5, 1.5, 4.5 ml/kg bw	60	Effect on liver and haemopoietic system	Gupta et al. (1998)
Azadirachtin	Rats	0.1 LD_{50}	42	Effect on blood constituents	Radwan et al. (2001b)
Azadirachtin	Rats	5000, 500–1500 mg/kg bw	1, 90	No toxicity	Raizada et al. (2001)
Nimbidin	Dogs, mice, rats	20–2000, 25–100, 10, 20 mg/kg bw	1, 42, 28	No toxicity	Pillai and Santhakumari (1984b)

value) was 79.4 mg/kg bw in rats (Pillai and Santhakumari, 1981).

The limonoids nimbolide and nimbinin showed in vitro activity against *Plasmodium berghei*, with an ED_{50} for nimbolide of 135 mg/kg bw/day (Bray et al., 1985). In vivo in mice no anti-malarial activity was observed (Bray et al., 1990).

6.2.2. Subacute effects

In rats, azadirachtin caused an increase of the albumin content, the blood glucose level and protein content. The red blood cell content was not affected, but the white blood cell content and platelet counts increased (Radwan et al., 2001a).

After rats had been administered azadirachtin during pregnancy (days 6–15) no adverse embryo/fetotoxicity and teratogenic effects or effects in reproductive parameters were found. The total number of implantations, post-implantation loss and foetal weight were not altered and there were no malformations due to the treatment (Srivastava and Raizada, 2001). Subacutely, nimbidin provided a protective effect against ulcers in induced gastric and duodenal lesions in rats and guinea pigs. It enhanced the healing process in acetic acid-induced chronic gastric lesions in rats and dogs (Pillai and Santhakumari, 1984a).

6.2.3. Semi-chronic effects

When rats were administered azadirachtin at high dose, a decrease in body weight gain and relative liver weights of rats was observed. There were decreases in TEC, Hb, erythrocyte sedimentation rate (ESR), PCV, and TLC. Serum protein, albumin and creatinine were lowered, SGOT increased, but no effect was found on blood urea nitrogen and SGPT. Histopathologically non-specific generalised degenerative changes were found. Thus, the formulation led to adverse effects on the haemopoietic system (Gupta et al., 1998). Azadirachtin in rats caused an increase of the blood urea content and in uric acid followed by a decrease to the normal rate at high concentration (Radwan et al., 2001b).

There was no toxicity of azadirachtin in rats even at 5 g/kg bw. Body weight, vital organs, enzyme activities in liver and serum and blood parameters did not change due to the treatment (Raizada et al., 2001).

Administration of nimbidin to rats, mice and dogs did not produce any signs of toxicity, although a dose-related weight gain, an increase in Hb level, an increase in liver glycogen and a reduction in serum protein were observed. Teratogenic studies in rats did not reveal any toxic manifestations or foetal abnormalities (Pillai and Santhakumari, 1984b).

6.3. Evaluation of the data

The best-documented pure compound from the neem tree is azadirachtin, which is present in leaves at an estimated concentration of 1.5 g/kg (Oguge et al., 1997) and in kernels up to 9 g/kg (Ascher, 1993). From one kilo kernels, by hand 100 ml oil can be extracted (Anonymous, 1995) and

other extraction methods yielded even 200 g oil/kg kernels (Saxena, 1989). If azadirachtin is retained in the kernel oil and is completely extracted, the concentration would be up to 45–90 g azadirachtin/kg oil. Toxicity was not found after administration of 5 g azadirachtin/kg bw in rats (Raizada et al., 2001).

When administered chronically, the non-toxic dose of azadirachtin was 1.5 g/kg bw in rats (Gupta et al., 1998; Raizada et al., 2001). From the NOAEL a safe chronic dose for human consumption of $1.5 \times 0.1 \times 0.1 = 0.015$ g azadirachtin/kg bw can be derived.

Taking into account that a LOAEL of 2.0 ml oil/kg bw was reported for the antifertility effect of neem oil in female rats, and that neem oil is known to contain about 45–90 g of azadirachtin/kg, it can be calculated that this 2.0 ml oil/kg implies exposure to about 0.09–0.18 g of azadirachtin/kg bw. This is far below the reported NOAEL of 1.5 g azadirachtin/kg bw. This leads to the conclusion that the toxic effects of neem oil are unlikely to be caused by its azadirachtin content.

7. Neem-based commercial-products

In some countries, selling neem-based products or pesticides on the market is allowed. This includes products like Praneem, a purified seed extract, and Ectozee.

7.1. Effects on humans

Little is published about the effect of neem-based products on humans. Only for Praneem, a purified seed extract, some studies were published as summarised in Table 9. A Praneem cream was developed which was devoid of irritation and sensitisation potential, as tested with rabbits and in a 21-day test on skin sensitivity in human volunteers. Talwar et al. (1995) investigated the toxicity aspects of Praneem as a contraceptive. There were no immediate or delayed reactions to the treatment. Haematological and biochemical parameters stayed within normal limits.

7.2. Effects on animals

Data on effects of neem-based products and pesticides on animals are summarised in Table 10 and discussed in the next paragraphs.

7.2.1. Praneem

The formulation of Praneem cream was safe in subacute toxicity studies in monkeys. The cream had a high contraceptive efficacy in rabbits and monkeys after intravaginal application (Garg et al., 1993b). The minimum effective spermaticidal concentration for Praneem was 25%. At this concentration, 100% of the sperm were immobilised within 20 s (Garg et al., 1994).

Oral treatment with Praneem of pregnant baboons and bonnet monkeys resulted in termination of pregnancy, without further effects, behavioural changes or alteration in food intake in the pregnant females themselves. Blood biochemistry and liver function were not altered and the treated animals regained normal cyclicity upon cessation of treatment and gave birth to normal offspring later (Mukherjee et al., 1996b). Administration of Praneem to pregnant rats caused complete resorption of the developing embryos on day 15 of pregnancy. The effect of the treatment was reversible and animals regained fertility. On administration, serum levels of T-$_H$1 cytokines (γ-interferon and tumour necrosis factor) were raised, which may be the cause of pregnancy termination (Mukherjee and Talwar, 1996).

7.2.2. Ectozee

High doses of Ectozee in rats led to anorexia, enlargement of the abdomen, drowsiness, tetanic spasms and haemorrhagic diarrhoea mostly resulting in death (Das, 1999).

7.2.3. Ruchamax

After treatment of anorectic goats with Ruchamax, appetite was restored. The rumen motility and the total bacterial and protozoal counts increased after treatment (Phalphale et al., 1997).

7.2.4. Nimbokil

At low doses, Nimbokil had no adverse effects on mice and did not reduce their fertility. The LD_{50} value was 16 ml/kg bw. Upon autopsy, no gross changes were seen in heart, lungs, liver, kidneys, ovary and testicles of the test animals. However, the product had a depressant effect on the CNS which, at higher concentrations, was causing death (Kazmi et al., 2001).

7.2.5. Vepacide

Long-term administration of Vepacide to rats caused dose-dependent loss in body weight and food intake, dullness, irritation, diarrhoea and weakness. Biochemical studies showed a dose- and time-dependent increase in SGPT and SGOT levels in serum, kidney and lung while these enzymes decreased in the liver. This profile indicates necrosis of the liver, and an adaptive mechanism in the other tissues due to the chemical stress. Lungs, liver and kidneys were most affected by the treatment (Rahman et al., 2001).

Table 9
effect of neem based products on humans

Product	Administration	Dose	Duration	Observed effect(s)	Reference
Praneem	In vitro	250 g/l	Once	Spermaticidal effect	Garg et al. (1994)
Praneem Vilci	Intra-uterine	1.5, 2, 2.5, 3 ml	3 days	Safety aspects	Talwar et al. (1995)

Table 10
Effect of neem based products on animals

Product	Test animal	Dose	Duration (days)	Observed effect(s)	Reference
Acute					
Praneem	Rabbits, monkeys	0.5 ml/day	21	No skin sensitivity	Garg et al. (1993b)
Praneem	Rats	0.6 ml/rat	3	Abortive effect	Mukherjee and Talwar (1996)
Praneem	Baboons, monkeys	3, 6 ml/animal	6	Embryo resorption	Mukherjee et al. (1996b)
Ectozee	Rats	0.1–0.5 ml/rat	1	Toxicity	Das (1999)
Hepatogard	Rats	650 mg/kg bw	1	Hepatoprotective effect	Rao et al. (1993)
Ruchamax	Goats	5000 mg/goat, twice daily	3–5	Anti-anorectic effect	Phalphale et al. (1997)
Subacute					
Nimbokil-60	Mice, rabbits	1–10 ml/kg bw, 0.0025, 0.005, 0.01 ml/kg bw	1, 28	Acute and subacute toxicity, effect on fertility	Kazmi et al. (2001)
Semi-chronic					
Vepacide-Tech	Rats	80, 160, 320 mg/kg bw	90	Toxicity	Mahboob et al. (1995)
Vepacide	Rats	80, 160, 320 mg/kg bw	90	Effect on enzyme profiles	Rahman et al. (2001)
Vepacide-Tech	Rats	1.0, 1.5, 2.0 g/kg bw; 80, 160, 320 mg/kg bw	1, 90	Effects in organs, toxicity	Mahboob et al. (1998)
Tric Vet Care	Rats	0.5, 1.5, 4.5 ml/kg bw	60	Effect on blood constituents, effects in organs	Kataria et al. (1998)

Acute administration of 80–320 mg/kg bw Vepacide-Tech (12% azadirachtin) in rats resulted in 10–80% mortality. Given the low toxicity of azadirachtin, being without effect up to 5 g/kg bw exposure, this effect of Vepacide-Tech cannot be caused by its azadirachtin content. Upon chronic administration, the highest doses caused a decrease in the P450 concentration in liver and lungs and all doses affected the kidneys (Mehboob et al., 1998). The changes induced by Vepacide were reversible on cessation of treatment (Mahboob et al., 1995).

7.2.6. Tric Vet Care

In rats treated with Tric Vet Care, catalase activity of the red blood cells increased. At high doses, lipid peroxidation increased in the brain and total ATPases decreased in both brain and liver. The activity of Mg^{2+} ATPase increased in the liver while it decreased in the brain. In the liver, acetylcholinesterase increased, whereas in the brain it decreased. The product affected liver and brain functions, possibly through membrane alteration and it could influence the oxidant defence mechanism of red blood cells and brain (Kataria et al., 1998).

7.2.7. NeemazalTM-T/S

In a review on the health evaluation of NeemazalTM-T/S, this product did not have any effect on reproduction, and did not cause skin or eye irritation (Niemann and Hilbig, 2000). LD_{50} values in several test animals were higher than 2 g/kg bw. No carcinogenicity was observed and 100 ppm did not have any effect (NOAEL) after 90 days administration in rats.

7.3. Evaluation of the data

Most of the neem-based products are toxic. For Praneem and Nimbokil-60 effects on reproduction and fertility are reported. Only Ruchamax and Hepatogard had positive effects in short term studies and for Neemazal LD_{50} values were higher than 2 g/kg bw. All other agents, administered once or chronically, negatively influence animal health and in some cases even cause death with medium lethal concentration (LC_{50}) values varying in the range from 1.6 to 16 ml/kg bw. The chemical composition of the commercially available agents is not always stated in the publications and the safe levels of ingestion as compared to the crude neem products and/or the various neem-based extracts and oils cannot be estimated.

8. Possible adverse health effects of neem applied as an insecticide

For the negative effects measured as a consequence of treatments, the NOAEL or, if that is not available from the references the LOAEL for unprocessed neem material, neem oil, aqueous and non-aqueous neem extracts and pure neem compounds are summarised in Table 11. There is no obvious trend for the values depending on the period of administration; chronic NOAEL's are not always lower than acute ones. The seed oil and the pure compounds are less toxic than unprocessed material or extracts. From this summary, it appears that aqueous extracts are roughly as toxic as non-aqueous extracts.

For all preparation methods, both toxic and beneficial effects were reported for which the applied dose does not seem the distinguishing factor; in some cases, the toxic effects are found at lower doses than the positive effects. The amount of active ingredients in the preparation might be influenced by the origin of the neem material used (Ascher, 1993), the extraction or preparation procedure, or the time between preparing and applying the agent. For calculations on the risk of pesticide residues, the effects of high temperatures

Table 11
Overview of NOAELs or LOAELs for non-aqueous and aqueous neem extracts, unprocessed neem material, neem oil, and the pure neem compound azadirachtin

	Reference	Effect	Dose	Test animal	Parameters measured
Unprocessed material					
Subacute	Kasturi et al. (1995, 1997)	LOAEL	80 mg/kg		Male fertility
	Parveen et al. (1993)	LOAEL	80 mg/kg		Male fertility
Semi-chronic	Aladakatti et al. (2001)	LOAEL	500 mg/kg	Rabbit	Male fertility
Seed oil					
Acute	Gandhi et al. (1988)	NOAEL	5 ml/kg	Rats	Toxicity
	Tandan et al. (1995)	NOAEL	7.4 g/kg	Mice	Toxicity
Subacute	Dhaliwal et al. (1998)	LOAEL	2 ml/kg		Female fertility
Semi-chronic	Chinnasamy et al. (1993)	NOAEL	10 g/kg		Toxicity
Aqueous extract					
Acute	El Hawary and Kholief (1990)	LOAEL	200 mg/kg	Mice	Toxicity and anti-diabetic effect
Subacute	Panda and Kar (2000)	NOAEL	40 mg/kg	Mice	Thyroid function
	Ray et al. (1996)	NOAEL	30 mg/kg		Immune response
Semi-chronic	Ali (1987)	LOAEL	50 mg/kg	Goat	Toxicity
Non-aqueous extract					
Acute	Singh et al. (1987)	LOAEL	50 mg/kg	Mice	Neuropsychopharmacological effect
Subacute	Choudhary et al. (1990)	LOAEL	100 mg/kg		Effect on reproduction
Semi-chronic	Kataria et al. (2000)	LOAEL	72 mg/kg		Toxicity
Pure compound (azadirachtin)					
Subacute	Srivastava and Raizada (2001)	NOAEL	1500 mg/kg	Rats	Fetotoxicity, teratogenicity
Semi-chronic	Raizada et al. (2001)	NOAEL	1500 mg/kg	Rats	Toxicity

during cooking of treated products on the active ingredients of neem are still to be examined. Therefore, the calculations presented here cannot be precise and safety should be better investigated, but the calculated safe doses give at least an indication of the risk one might run upon ingestion of neem or neem treated products.

8.1. Unprocessed material

The risk of ingestion of unprocessed neem products as pesticide residues on beans can be calculated as follows. A generally used dose of neem leaf powder for the protection of stored beans against insects is 25 g/kg seeds (see Boeke et al., 2001 for an overview). A daily meal of 150 g beans, presuming no effect of washing, would then contain maximally 3.75 g of powder residue. This dose is about 200 times higher than the calculated safe dose of 18.5 mg in Section 2.3. However, most of the powder is easily sieved off or removed after washing of the treated seeds with water.

8.2. Seed oil

Neem oil is generally applied at about 5 ml/kg beans, which, in a meal of 150 g beans would leave a residue of 0.75 ml. This is about five times higher than the estimated safe dose of 0.14 ml oil calculated in Section 3.3, but it is far lower than the doses of 5 ml shown to be toxic upon ingestion by small children (Section 3.1).

8.3. Aqueous extract

The effects of aqueous extracts on animals are ambiguous, as is their use on stored seeds. Stored seeds should be kept as dry as possible to prevent moulds causing mycotoxin contamination and early germination (De Groot, 1991). No safe dose can be proposed here.

8.4. Non-aqueous extract

For non-aqueous extracts, once the solvent has evaporated, the doses of neem material on the seeds will be low. However, the exact dose is difficult to estimate. No reliable estimations can be made on the risks caused by exposure to these residues.

8.5. Pure compound

Pure neem compounds, especially azadirachtin, when calculated relative to the quantities of crude material, could be called non-toxic. For neem-based pesticides, a range of tests should be performed before their commercial release. The effects and safe use and dose should be mentioned on the packages.

8.6. Evaluation of the data

From the rough risk assessments presented in this study, the use of neem-based products as an insecticide to protect

Table 12
Overview of the estimated safe daily dosis for unprocessed neem material, neem oil, aqueous and non-aqueous neem extracts, and the pure neem compound azadirachtin

Neem material	Estimated safe daily dosis
Unprocessed material (mg/kg bw)	0.26
Oil (μl/kg bw ~mg/kg bw)	2
Aqueous extract (mg/kg bw)	0.3
Non-aqueous extract (μg/kg bw)	12.5
Pure compound (azadirachtin) (mg/kg bw)	15

stored seeds for consumption, if applied with care, should not be discouraged. Studies are needed to assess the exposure using different preparations.

At the indicated doses, most of the neem preparations are effective against insects and the advantages of keeping the stored seeds in a good condition at low cost would outweigh the possible disadvantageous effects of the treatment.

9. General conclusion

In this paper the safety evaluation of neem-based products refers to the use of neem as a pesticide. In order to enable a comparison of the relative health risks of the different neem-based products, the calculated or estimated safe doses of daily intake of each product are given (Table 12).

From all the neem-based products described and evaluated in this review, the pure compound azadirachtin, the unprocessed materials, the aqueous extracts and the seed oil are the most safe to use as an insecticide to protect stored consumption seeds for human consumption (estimated safe daily doses of 15, 0.26 and 0.3 mg/kg bw,2 μl/kg bw/day respectively). On the other hand non-aqueous extracts turn out to be relatively toxic (estimated safe daily doses of 12.5 μg/kg bw). As the toxicological profile and the composition of the different products and fractions are unknown, it is difficult to conclude about the toxic principles underlying the toxicity data. It is suggested that other compounds than azadirachtin are responsible for the toxic effect for the non-aqueous extracts. For the non-aqueous extract the estimated safe daily dose is only based on the no adverse effect level obtained from acetone-extracts. Therefore, further analysis of the toxic components is needed to arrive at more definite and reliable conclusions on the safe intake of preparations and extracts. Furthermore, aflatoxin concentrations in neem are not properly controlled posing an additional risk factor.

Data from literature and from human experience both suggest that the application modes most feasible for low-resource farmers in tropical countries are also the most advisable from a health risk viewpoint.

Concerning the possible adverse effects the most critical ones are reproduction disturbances, although these are often reversible.

We recommend that if applied with care the use of especially unprocessed and aqueous neem-based products should not be discouraged.

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