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Ricin (from *Ricinus communis*) as undesirable substances in animal feed[1]

Scientific Opinion of the Panel on Contaminants in the Food Chain

(Question N° EFSA-Q-2003-062)

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SUMMARY

Ricin is a toxic glycoprotein (with several minor variants) belonging to the type II group of ribosome inactivating proteins (type II RIP) found in the seeds (beans) of the castor oil plant (*Ricinus communis L.* (Euphorbiaceae)). It is composed of two polypeptide chains of approximately 30 kDa joined by a disulfide bond. A limited number of other plants in the same family contain type II RIPs, *i.a.* subtropical leguminous climber *Abrus precatorius* L. and, *Croton*

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tiglium L. which contain abrin and crotin I, respectively. The seeds of *Croton tiglium* contain a number of other toxins which make it unsuitable as a feed for livestock. In the Terms of Reference, the plant *Jatropha curcas* was also requested to be considered, however, it does not contain a RIP II protein. The toxicity of its seeds can be ascribed to the oil, which contain phorbol esters and this plant is therefore not relevant for this opinion on ricin.

Following extraction of castor oil, ricin is left in the press-cake/castor bean meal[2]. Castor oil production mainly takes place outside the EU. Because of its low value of the press-cake as feed no import to the EU is expected.

Following cell uptake by endocytosis, ricin causes acute cell death by inactivation of ribosomal RNA. Acute symptoms in humans after intake of castor beans are hematemesis (vomiting containing blood), diarrhoea, haemorrhagic necroses in several organs, renal failure, circulatory collapse and death after 6 to 14 days with a fatal oral dose of about 1 mg/kg b.w. (5-10 castor beans). Because of its destruction in the intestinal tract, ricin is approximately 1000-fold more toxic following parenteral administration or inhalation, than by the oral route. Oral LD_{50} values in rats and mice were 20 to 30 mg/kg b.w., and the corresponding intra peritoneal LD_{50} value for mice is 22 µg/kg b.w. There are no data on chronic or reproductive toxicity, or genotoxicity of ricin. Crotin I showed LD_{50} *i.p.* values in mice of 20 mg/kg b.w.

The very limited data on acute toxicity in target animals comprise mainly information on castor bean products rather than on purified ricin. Amongst ruminants, cattle appear to tolerate higher intakes than sheep. In horses severe colic and death have been observed after a single dose of approximately 7-8 mg ricin/kg b.w. Toxic effects in pigs and birds have been reported as well as accidental poisonings in dogs with vomiting, depression and diarrhoea as the main clinical signs. No- or lowest observed adverse effect levels (NOAELs or LOAELs) for acute effects of ricin could not be identified for any of the animal species.

The Panel on Contaminants in the Food Chain (CONTAM Panel) is not aware of any feed producers using castor seed meal as a feed for livestock in the EU, and therefore subject to this caveat, exposure of animals would only be expected as a result of accidental contamination. There are limited data on the toxicokinetics and carry-over of ricin to products of animal origin (milk, meat or eggs). Livestock has a very low tolerance to ricin exposure via feed before clinical symptoms of toxicity are manifest. It is therefore unlikely that highly exposed animals would enter the food chain, and the CONTAM Panel considers the risk of ricin transfer to livestock products to be negligible.

[2] Castor bean meal is also called castor meal, castor residue, castor extract and de-oiled castor cake

Except for exposures to ricin via the accidental intake of castor beans (*Ricinus communis*) human exposure via food, under normal circumstances, is unlikely to occur.

KEYWORDS: Ricin, *Ricinus communis*, *Croton tiglium*, Euphorbiaceae, *Jatropha curcas*, Crotin, castor oil seeds, castor meal, RIP II protein, toxicity, exposure, carry-over, animal health, human health.

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1. General background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed[3] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[4].

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination ("early warning system") and to take measures to reduce or eliminate the contamination ("pro-active approach").

In particular the introduction of the principle of non-dilution is an important and far- reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful mean to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated

[3] OJ L140, 30.5.2002, p. 10
[4] OJ L 115, 4.5.1999, p. 32

scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)[5].

It is worthwhile to note that Council Directive 1999/29/EC is a legal consolidation of Council Directive 74/63/EEC of 17 December 1973 on the undesirable substances in animal nutrition[6], which has been frequently and substantially amended. Consequently, several of the provisions of the Annex to Directive 2002/32/EC date back from 1973.

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003[7] provides a comprehensive overview on the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health that additional detailed risk assessments are necessary to enable a complete review of the provisions in the Annex.

2. Specific background

The castor oil plant (*Ricinus communis L.)*, belonging to the plant family of *Euphorbiaceae*, is highly toxic because of the presence of ricin, a water-soluble glycoprotein concentrated in the seed endosperm but present in lower amounts in the rest of the plant and reputed to be one of the most poisonous of the naturally occurring compounds. All animals (livestock and pets) are vulnerable. Analytical methods exist for the direct determination of ricin.

The castor oil plant – *Ricinus communis* is listed as an undesirable substance in the Annex of Directive 2002/32/EC. A maximum level of 10 mg/kg, expressed in terms of castor-oil plant husks is established for all feedingstuffs

SCAN concluded[8] that insufficient data are available to consider a full risk assessment of botanical contaminants. However, risk assessments could be made for some of the compounds presumed responsible for their toxicity and consequently maximum limits for botanical contaminants of particular concern should be set on the basis of their known toxicants. SCAN

[5] Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)
[6] OJ L 38, 11.2.1974, p. 31
[7] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)
[8] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 9.5. Conclusion and point 9.6 Recommendations.

recommends that priority should be given to ricin (from *Ricinus communis)* or a marker of ricin. In the meantime, any detection of *Ricinus communis* should lead to rejection of the feed material.

SCAN refers in its opinion also the botanical impurities *Jatropha curcas L.* (purghera, physical nut) and *Croton tiglium L.* (croton)[9] both listed in the Annex to Directive 2002/32/EC.

Jatropha curcas is another member of the family *Euphorbiaceae* and known for its toxicity. The seeds contain curcin, a toxic glycoprotein with a 54% homology with the ricin A chain and with similar mode of action.

Croton tiglium is also member of the family *Euphorbiaceae* but somewhat less toxic than *Ricinus* and *Jatropha*. The major toxic agent, croton, is a collective name for a group of seed glycoproteins each with varying toxicity but with similar mode of action to that of ricin.

Given their similar mode of action as ricin, it should be considered if a risk assessment on ricin should also include plant toxins with similar mode of action such as curcin and croton.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the EFSA to provide a scientific opinion on the presence of ricin (from *Ricinus communis)* in animal feed.

This scientific opinion should comprise the

- evaluation if it is appropriate that the risk assessment on ricin *(Ricinus communis)* includes also plant toxicants with a similar mode of action such as curcin (from *Jatropha curcas)* and crotin (from *Croton tiglium*) or if for these plant toxicants separate risk assessments have to be performed.
- determination of the toxic exposure levels (daily exposure) of ricin (and eventually curcin and crotin) for the different animal species of relevance (difference in sensitivity between animal species) above which
    - signs of toxicity can be observed (animal health / impact on animal health)

[9] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, points 9.2.2. and 9.2.3.

  - level of transfer/carry over of ricin (and eventually curcin and crotin) from the feed to the products of animal origin results in unacceptable levels of ricin (and eventually curcin and crotin) and/or eventually their toxic metabolites in the products of animal origin in view of providing a high level of public health protection.
- identification of feed materials which could be considered as sources of contamination by ricin (from *Ricinus communis)*(and eventually curcin and crotin) and the characterisation, insofar as possible, of the distribution of levels of contamination
- assessment of the contribution of the different identified feed materials as sources of contamination by ricin (and eventually curcin and crotin)
  - to the overall exposure of the different relevant animal species to ricin (and eventually curcin and crotin),
  - to the impact on animal health,
  - insofar relevant, to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry over rates.
- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

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ASSESSMENT

1. Introduction

The castor oil plant (*Ricinus communis L.*) is a member of the Spurge family of plants (Euphorbiaceae). It is grown commercially for the oil contained in the seed, which is used

primarily for industrial purposes and in the manufacture of cosmetics. Most of the world's castor oil is produced in India, China and Brazil, but commercial production also occurs on a smaller scale in many other tropical countries. World production of castor oil increased from 0.4 million tonnes in 1970 to 0.8 million tonnes in 2000 (Weiss, 2000). Most of the global oil extraction occurs in the countries in which it is produced. The EU uses 10,000 tonnes of castor oil annually (Anonymous, 2006), of which approximately 8,000 tonnes of castor oil are produced in the EU[10]. Therefore, only small quantities of castor bean meal (<5,000 tonnes) are produced in the EU, and there are no data on the extent of use of this meal, e.g. as a feed or fertilizer.

The oil makes up about 50% of the weight of the seeds. The oil is mostly constituted of ricinoleic acid, with small amounts of dihydroxystearic, linoleic, oleic, and stearic acids. The main uses of castor oil include the industrial production of coatings based on dehydrated alkyd resins, but it is also used in the manufacture of pharmaceuticals and cosmetics and as a laxative (Cosmetic Ingredient Review Expert Panel, 2007), in the textile and leather industries, and for manufacturing plastics, fibres soaps, printing inks, wetting agents, and lubricants (Weiss, 2000).

The toxic glucoprotein ricin is not transferred to the oil fraction during extraction, which today in general is done by cold pressing the seeds (Cosmetic Ingredient Review Expert Panel, 2007), but remains in the seed cake. Therefore the seed cake must be detoxified before used as an animal feed. A number of methods have been employed to detoxify castor oil seed meal, some of which appear to be more effective than others (Okorie *et al.*, 1985; Rao *et al.*, 1988; Puttaraj, 1994).

Plant breeders in the United States are currently utilising biotechnology to develop a ricin-free castor bean plant[11]. Elimination of the toxin would enable the castor seed and castor bean meal to be used as an animal feed without the necessity of detoxification, thus increasing the economic value of the crop.

Croton tiglium L. (Euphorbiaceae) is a small, erect tree that will thrive on poor soils. It is native in South East Asia, but it has been introduced into West Africa. The plant contains toxins, particularly in the seeds. For a man about four seeds, and for a horse about 15 seeds, represent a lethal dose. In view of the presence of these toxins, croton seed cake is not suitable for use as a feed for livestock (Pettit, 1977). It was used for the production of oil by pressing or extracting the seeds with ether. The oil is a very strong laxative (a violent purgative) and is highly toxic when used as such (15 drops stated to be a fatal dose for a man). The oil has also been used in preparations as a counterirritant on the skin (Buchheister and Ottersbach, 1919). However, it easily causes pustular eruptions when applied to the skin (Tyler *et al.*, 1976). Later the oil was deemed unsafe for either use due to its content of cocarcinogenic activity (Hecker, 1968). The

[10] Fediol - EC Seed Crushers' and Oil Processors' Federation, 2004
[11] Arcadia Biosciences, Phoenix, Arizona

press or extraction cake contains the toxic protein crotin I which may present a risk to animals if found in feeding stuffs (Sperti *et al.*, 1976).

Jatropha curcas is a tree belonging to the *Euphorbiaceae* family. It originated in Central America, but is now found in many tropical and sub-tropical countries in Africa and Asia. The seeds contain 28-40% oil. For many years the oil was used predominantly in the manufacture of soaps and candles, but more recently Jatropha oil has become of significant economic importance as a result of its potential as a source of biodiesel. Jatropha seed-cake contains toxins, making it unsuitable for animal feed. However, it does have potential as a fertilizer, and if available in large quantities, it can also be used as a fuel for steam turbines to generate electricity. A method has been developed for detoxifying the press-cake through a combination of heat treatment and solvent extraction, but it was not an economically viable option for commercial production (Benge, 2006; Martinez-Herrera *et al.,* 2006).

It is not known if the seeds of *Ricinus communis, Croton tiglium or Jatropha curcas* have been found as botanical impurities in other feed materials.

1.1. Bioactive compounds

Castor plant

The castor plant contains in its seeds (sometimes named beans[12]) a group of closely related toxic glycoproteins (the ricin group), ricinoleic acid (12-hydroxyoleic acid) and the alkaloid ricinin. The latter compound is often used as an indicator of the presence of material from castor beans in press cakes in feeding stuffs (Darby *et al.*, 2001).The seed oil was, especially earlier, used as a laxative/purgative due to the direct effect of the ricinoleic acid on the small intestine. The free acid is released by enzymatic hydrolysis in the intestines from the lipids in which is makes up around 70% of the fatty acid residues (Hänsel and Hass, 1983).

The toxicity of Ricinus seeds has been recognised since ancient times, and its toxicity to humans has recently been reviewed (Olsnes, 2004; Audi *et al.*, 2005). Castor beans were used in the classical Egyptian and Greek medicine, and were described in the Sanskrit work on medicine, *Susruta Ayurveda* from the sixth century B.C. (Olsnes, 2004). Intoxication by castor beans is not rare in countries where the plant grows in abundance, and by 1974 at least 700 cases of human intoxication had been described (Balint, 1974).

[12] In this opinion the terms seed or bean are used according to the original publications

More than a century ago, Stillmark isolated a toxic protein from the seeds, which he termed ricin (Stillmark, 1888). At that time the toxicity was believed to result from the observed ability of the ricin preparation to agglutinate blood cells. More recent studies established that Stillmark's ricin preparations were a mixture of two proteins, namely the potent cytotoxin (ricin) and a haemagglutinin (Lord *et al*., 1994).

The term ricin, as used in this opinion, covers a group of very closely related glycoproteins, which consist of two polypeptide chains each of approximately 30 kDa and joined by a disulfide bond (Lord *et al*., 1994; Frederiksson *et al.,* 2005). One of the chains (chain A) belongs to the group of ribosome inactivating proteins (RIP I proteins) because of their ability to inactivate cell free ribosomes from eukaryotes (Roberts and Smith, 2004). The second (chain B) is a sugar-binding protein. Such dimeric and toxic compounds together are designated type II RIPs (Lord *et al.*, 1994). Ricin is synthesized in the castor bean endosperm (Lamb *et al.*, 1985; Frigerio *et al.*, 1998, 2001).

Many proteins structurally and functionally related to that of the A chain of ricin have been characterised from a wide variety of plants. These proteins called type I RIPs usually occur as monomers of approximately 30 kDa. However, in plants such as *Ricinus communis, Abrus precatorius* L (Fabaceae) and *Croton tiglium* L., the covalent binding of the RIP I (chain A) to the sugar-binding chain (chain B) produces a RIP II molecule, which is cytotoxic because the addition of chain B facilitates the uptake of the protein into mammalian cells.

As such, ricin and other type II RIP's are members of the A-B family of toxins that also comprises several bacterial toxins, such as diphtheria, cholera, Shiga and anthrax toxins (Olsnes *et al.*, 1999).

Ricin contains multiple tryptophans (especially in chain B); but it has not been well understood how the aromaticity of tryptophan influences the physical characteristics of ricin in varying environments. Significant changes in the properties of ricin, such as affinity for galactosides, have been observed at lower values of pH (Frenoy, 1986). A number of physiochemical and photochemical characteristics of ricin have recently been reviewed (Gaigalas *et al.*, 2007).

According to Lin and Li (1980) there are two toxic ricins: ricin D and E. Ricin E appears to be present in small grain castor bean plant types (Woo *et al.*, 1998).

While the so-called ricin D binds to Sepharose (by the use of which it traditionally has been purified through affinity chromatography) the other type, designated ricin E, does not (Woo *et al.*, 1998).

Ricin D as the most investigated ricin has been shown to be a glycoprotein containing two lactose moieties in the B-chain and one in the A-chain (Kozlov *et al.*, 2006; Montfort *et al.*, 1987; Wales *et al.*, 1991; El-Nikhely *et al.*, 2007).

Traditionally, the difference in toxicity between the two types of ricin has been ascribed to a difference between the two B chains, resulting in different affinity to saccharides. Woo *et al.* (2001) also showed that the A chains from ricin E and D differ; i.e. they are not identical in terms of the molecular mass and isoelectric point, indicating that ricins consist of many chain A variants, as well as variants of the chain B (Woo *et al.*, 2001).

The three-dimensional structure of ricin and its chains has been elucidated by X-ray crystallography (Montfort *et al.*, 1987; Rutenber *et al.*, 1991; Katzin *et al.*, 1991; Rutenber and Robertus, 1991).

In addition to ricin, the seeds of *R. communis* also contain the *Ricinus communis* agglutinin (RCA). Together these compounds make up around 5% of the total protein in the mature seeds. RCA is a tetrameric protein with stronger agglutinating properties than ricin but is less toxic (Lord *et al.*, 1994; Bigalke and Rummel, 2005).

Other plants

A limited number of other plant species have been shown to contain type II RIP's, which make them potentially toxic to animals and to man. These include the subtropical leguminous climber *Abrus precatorius* L. reported to provide fibres and to formerly being used as a poison containing abrin (Lock, 1989; Bagaria *et al.*, 2006) and, *Croton tiglium* L. containing crotin II (Stirpe and Battelli, 2006). Other compounds with type II RIP properties include Mistletoe lectin I from *Viscum album* leaves, Modeccin from *Adenia digitata* root, Volkensin from *Adenia volkensii* root, RIP from *Adenia goetzii* caudex, Lanceolin from *Adenia lanceolata* caudex, Stenodactylin from *Adenia stenodactyla* caudex, Aralin from *Aralia elata* shoots and Riproximin from *Ximenia americana* powder (Stirpe and Battelli, 2006).

Croton tiglium L. has long been known as a toxic plant. The seeds are particularly toxic, containing a number of skin-irritating phorbol esters and showing a strong laxative effect residing in the oil (croton oil) (Roth *et al.*, 1994). However, in addition to the phorbol esters the seeds also contain RIPs of both type I and II (known as crotin II and crotin I, respectively) (Stirpe *et al.*, 1976; Sperti *et al.*, 1976). Extracts from *Croton tiglium* inhibit protein synthesis in cell-free system. Crotin II had the greater effect *in vitro*, with inhibition of protein synthesis being of the same order as that obtained with pure ricin, although it was 1000 times less toxic than ricin *in vivo*. The molecular mass of crotin I is around 40 kDa, while that of crotin II is approximately 15 kDa as measured by SDS-PAGE (Chen *et al.*, 1993). In this respect the crotins differ from the

other known RIPs which in general show molecular weights of around 30 kDa and 60 kDa for the type I and type II compounds, respectively (Chen *et al.*, 1993).

The plant *Jatropha curcas* was previously believed to also contain RIP II (curcin) in its seeds as it has been reported to be toxic to humans, rodents and livestock (Heller, 1996; Ghandi *et al.*, 1995). However, more recent research has shown that curcin is a type I RIP being less toxic and point to the irritant phorbol esters as being the major class of toxins (Makkar and Becker, 1997; Becker and Makkar, 1998; Martinez-Herrera *et al.*, 2006; Barbieri *et al.*, 1993; Lin *et al.*, 2003). Reports on the intoxication of humans by accidental ingestion of the oil or seeds have appeared in Hawaii, Florida, Philippines and India (Kulkarni *et al.*, 2005). The symptoms of intoxication in humans include burning and pain in the mouth and throat, vomiting, delirium, muscle shock, decrease of visual capacity, and an increase in heart rate (Kingsbury, 1964). A high mortality rate has been reported for rodents (mice, rats) and domestic animals (sheep, goats, calves, and chicks) when fed *Jatropha curcas* seeds (Adam and Magzoub, 1975; Ahmed and Adam, 1979a,b; Liberalino, 1988; El Badwi *et al.*, 1992, 1995; Ghandi *et al.*, 1995). After expression, both the oil and the press cake are toxic (Stirpe *et al.*, 1976; El Badwi *et al.*, 1992). Phorbol esters are present in several *Jatropha* species and different provenances of *J. curcas* contain significantly different levels of these esters, some provenances being atoxic (Adolf *et al.*, 1984; Makkar *et al.*, 1997). Hence, this plant is not further discussed in this opinion.

In conclusion, *Ricinus communis* and *Croton tiglium* are relevant for the present risk assessment because of their content of potent RIP II proteins. While some indications as to the quantitative content of ricin in castor beans are available it should be noted that this is not the case for croton seeds. The toxicity of *Jatropha curcas* is not caused by RIP II proteins and hence is different from that of ricin in *Ricinus communis* and crotin I in *Croton tiglium*, and therefore not further discussed in this opinion. At present the available information on the toxic principles of *Jatropha curcas* is very limited and the Panel decided that a conclusive evaluation of these scarce data is not possible.

1.2. Hazard assessment for humans

The acute toxicity of ricin in humans and other mammals is due to its ability to inactivate ribosomes in cells.

The acute toxicity of ricin is very variable depending on the animal species, the strain and the route of exposure. Oral LD_{50}s in rodents have been reported in the literature with 20-30 mg/kg rats and 30mg/kg b.w. in mice (Audi *et al.*, 2005; Cook *et al.*, 2006). For the intravenous, inhalation, and intraperitoneal routes, toxicity is approximately 1000-fold higher than via the oral route with respective LD_{50} values of 2-10 μg/kg b.w., 3-5 μg/kg b.w, and 22 μg/kg, b.w. in mice

(Audi *et al.*, 2005; Kumar *et al.*, 2007; He *et al.*, 2007). The lower toxicity of ricin after oral exposure is due to the destruction of ricin in the lumen of the intestinal tract (Olsnes, 2004; Wedin *et al.*, 1986).

Most studies on ricin toxicity have been performed using *intra peritoneal (i.p.)* administration. Hepatotoxicity, nephrotoxicity, and oxidative stress following *i.p.* administration of high levels of ricin (25 μg/kg b.w.) have been investigated in Swiss albino male mice. Blood urea levels and activities of glutamate pyruvate transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, and lactate dehydrogenase were all increased in the plasma, the liver, and the kidney indicating damage in those organs. Markers of oxidative stress were also affected in liver and kidney: increase in lipid peroxidation and catalase activity, decrease of total non-protein sulfhydryl superoxide dismutase and glutathione peroxidase activities Overall, hepatotoxicity was more prominent than nephrotoxicity (Kumar *et al.*, 2003). Recently, DNA damage and oxidative stress have been observed after *i.p.* injection in mice from as early as 24 hours at a minimum dose of 1 μg/kg b.w. (Kumar *et al.*, 2007).

In female CF-1 mice, ricin showed similar effects after (25 μg/kg b.w.) *i.p.* administration of with a 2- to 3-fold increase in hepatic lipid peroxidation and in the incidence of hepatic DNA single-strand breaks and a decrease in non-protein sulfhydryl concentrations at 24, 36, and 48 hours post-treatment. Decreases in liver and intestinal weight to body weight ratios were observed in ricin-treated animals, while no changes were observed in spleen and kidney weight to body weight ratios. Overall, the authors concluded that the onset of ricin-induced hepatic oxidative stress was maximal at 36 hours post-treatment (Muldoon *et al.*, 1992).

After *i.p.* injection of 5 μg/kg b.w. in mice, an increase in the urinary excretion of oxidative stress markers (malondialdehyde, formaldehyde, and acetone) has been shown to be modulated through macrophage activation via tumour necrosis factor alpha (TNF-alpha). Indeed, treatment with a TNF-alpha antibody reduced the latter effects as well as hepatic lipid peroxidation, glutathione depletion, and DNA single-strand break production. The authors concluded that macrophage activation and subsequent release of TNF-alpha are involved in ricin toxicity (Muldoon *et al.*, 1994). Ricin (5 and 25 μg/kg *i.p)* induced a dose-dependent hepatic lipid peroxidation, which included microsomal, mitochondrial and macrophage superoxide anion production. This effect was abrogated by adding desferrioxamine to the microsomes. In contrast, Fe^{2+} treatment increased lipid peroxidation dramatically suggesting that iron mediated production of superoxide anion is also involved ricin-mediated oxidative stress (Muldoon *et al.*, 1996).

In rats, *i.p.* injection of 15 μg ricin/kg b.w. led to thyroid toxicity with necrosis of the follicles and the authors correlated these histopathological results with increased lipid peroxidation and a 50% reduction in circulating thyroid hormone levels (Sadani *et al.*, 1997*)*.

In humans, about 700 cases of intoxication from intake of castor beans have been reported, but only a few fatalities have been described. The symptoms consist of haematemesis, diarrhoea, and death occurred after 6 to 14 days as a result of dehydration, hypovolumemia and circulatory collapse (Doan, 2004; Rauber and Heard, 1985). The fatal oral dose of ricin in humans has been estimated to range from 1 (about 5 to 10 castor beans) to 20 mg of ricin/kg b.w. (Bradbury *et al.*, 2003; Audi *et al.*, 2005),.

One case of fatality (assassination) following intramuscular injection of a small ampoule putatively containing ricin has been described[13]. The person developed fever, glandular swelling, haematemesis (vomiting containing blood) and leukocytosis followed by renal failure and a clinical picture resembling sepsis and multi-organ failure. Upon autopsy haemorrhagic necroses were found in the intestinal wall, the heart muscle and in lymph glands. Similar clinical and pathological findings were observed in pigs injected with ricin. Inhalation of ricin has not been described in humans, but is probably the most toxic route of administration as shown in animals (LD_{50} in mice 3-5 µg/kg b.w). In experimental animals, inhalation results in inflammation in the bronchial tree and alveoli and lung oedema caused by injured endothelial cells. The symptoms are expected to develop during 8 hours and respiratory failure during 36 to 72 hours (Olsnes, 2004; Bismuth *et al.*, 2004).

There is no information on chronic toxicity, and there is also no information on reproductive toxicity and genotoxicity.

The mechanism of toxicity has been elucidated in great detail. In short, the galactose-binding part of the molecule (the B chain) binds to the target cell surface allowing ricin to enter the cell by endocytosis. Once taken up in the endosome some of the toxin is degraded in lysosymes, a second part is recycled to the cell surface. A minor part (about 5%) is translocated to the Golgi apparatus and is shuttled to the endoplasmic reticulum. Here several of the ricin molecules can be exported from the cell by exocytosis. From the endoplasmic reticulum the A chain of ricin is translocated to the cytoplasm by the mechanism used by misfolded proteins targeted for proteasomal degradation. In the cytoplasm the A chain of ricin acts as a glycosylase, which removes a highly conserved adenine from 28S ribosomal RNA, and thereby inhibits the protein synthesis. As the velocity of this reaction is much greater than the speed of re-synthesis/repair, in theory, one molecule of ricin would be enough to kill one cell. In order to enter the cell and translocate to the site of action as an undamaged A chain the molecule, however, also has to be able to circumvent a number of cellular defence mechanisms, and indeed ricin does possess

[13] Bulgarian dissident Georgi Markov was killed by a poison dart filled with ricin and fired from an umbrella in London in 1978.

several unique features to overcome such mechanisms (Olsnes, 2004; Utskarpen *et al.,* 2006; Lord *et al*., 1994; Endo and Tsurugi, 1987; Endo *et al.,* 1987; Parikh *et al.,* 2008).

Comparison of the *in vitro* cytotoxicity in cell cultures of ricin E and ricin D shows that ricin E is somewhat less toxic than ricin D (Woo *et al.,* 1998).

Crotin I

Crotin I was shown to be moderately toxic to mice when given *i.p.*, with LD_{50} values of 0.45 mg/mouse (corresponding to about 20 mg/kg b.w.) (Stirpe *et al.*, 1976; Chen *et al.*, 2003).

2. Methods of analysis

Since 1980 and to the present day, not more than around 25 papers on chemical quantitative analysis of ricin have been published internationally in the open literature. This is in spite of the fact that ricin is the only protein listed under the Chemical Weapons Convention[14] and as such is of great interest concerning advanced developments. Chemical quantification is clearly not without its difficulties.

Usually proteineous toxins like ricins and related compounds described above, are determined using conventional methods for proteins, such as immunochemical methods of various kinds e.g. electrophoresis. While electrophoresis may be a method of potential use, only one reference to this method has been published. This demonstrated the potential for the use of capillary electrophoresis, although only for samples with a relatively high content, such as extracts of castor been seeds, which produced sufficiently large peaks to allow identification and semi-quantification (Hines and Brueggemann, 1994).

Although, the ricin D and E forms are different analytes, these are not usually determined separately.

Chemical methods

Another approach has been taken to detect and determine ricins and marker compounds for the presence of material from *Ricinus communis.* The general protein identification scheme using mass spectrometry is based on LC/MS or MALDI-TOF for size classification followed by the use of the same instrumentation for the analysis of the tryptic digest. Fragments of the digest can be searched in a database for tentative identification of the unknown protein and then followed by comparison to authentic reference material. Using this method Darby *et al.* (2001) determined

[14] http://www.opcw.org/docs/cwc_eng.pdf

the content of ricin in castor beans, showing that the identification of the alkaloid ricinine by GC/MS and/or LC/MS can be a good complementary technique for the determination of castor bean extracts. Seto and Kanamori-Kataoka (2005) and Frederiksson *et al.* (2005) have further investigated the use of different MS methods in the identification of ricin in different samples, among others crude castor bean extracts (Frederiksson *et al.*, 2005).

Mass spectrometry has been used for the detection of the *R. communis* specific alkaloid ricinine (3-cyano-4-methoxy-N-methyl-2-pyridone) as a marker of contamination of feed with materials from *R. communis* (Darby *et al.,* 2001; Mouser *et al.,* 2007; Johnson *et al.*, 2005).

According to recent literature a ricin reference material that can be used to test detection devices for this chemical (and serious biological threat) is under development (Kim *et al.,* 2006).

Very few studies have been published describing the quantitative analysis of crotin I and crotin II. Yuan *et al.* analysed both crotins using high performance gel filtration chromatography (GFC) with a photodiode array detector after the crude extracts from Croton tiglium were separated on a Protein-Pak 60 + Protein-Pak 125 column using phosphate buffer (0.2 mol/L), pH 6.5. The purity of the constituents was assessed by their respective spectra. The molecular weights of crotin I and crotin II were estimated as 40 kDa and 19 kDa, respectively and the amino acid sequences of both crotins were determined with OPA-postcolumn derivatisation (Yuan *et al.,* 1994).

Biological methods

Most analytical methods for determination of ricin are immunological methods. These methods are based on monoclonal or polyclonal antibodies. Analyses seem not to have been performed on feed samples. The development of immunochemical methods faces the problem of how to produce antibodies to these extremely toxic compounds. This can be done by using formalin toxoided (inactivated) ricin (Cmiech *et al.,* 1985) and on this basis a number of more sensitive immunochemical assays for ricin have been developed, starting with the radioimmunoassay developed by Ramakrishnan *et al.* (1982) and the early developments within ELISA by Koja *et al.*, (1980). These methods were succeeded by a number of more sensitive ELISA methods (Griffiths *et al.*, 1986; Leith *et al.,* 1988). For all such methods it must be ensured that no co-determination of the chemically related *Ricinus communis* agglutinin (RCA) is occurring.

The method of Leith *et al.*, (1986), which works on the optimisation of the extraction of ricin achieves a recovery of 84% with a limit of detection of 20 pg of ricin per 100 μL of tissue extract. Later on, Poli *et al.* (1994) demonstrated a higher sensitivity using affinity purified polyclonal antibodies in combination with chemiluminescence detection. The assay was tested on human urine and human serum.

Later developments with the immunochemically based methods for quantification have focussed on such details as further work on the development of monoclonal antibodies (Dertzbaugh *et al.*,

2005) and the construction and test of sandwich immunoassays for the detection of several toxins (Goldman *et al.*, 2004) and a large number of biosensors (Shankar *et al.*, 2005; Rubina *et al.*, 2005; Dill *et al.*, 2004). While none of these have been developed for or used on feed materials many of them do represent potential methods for further development in this area.

Very recently it was demonstrated that ricin can be determined quantitatively by means of an enzyme linked immunosorbent assay (ELISA) double antibody sandwich method in matrices such as water, soil, milk powder and blood, with a limit of detection of around 0.2 μg/L in the extract to be analysed (Yang *et al.*, 2007).

A very sensitive immuno polymerase chain reaction (IPCR) assay was developed for the detection of type I and type II RIPs including ricin. It combines the binding of ricin with a specific antibody with amplified detection using polymerase chain reaction (PCR). The limit of detection (LOD) of the technique was compared with the LODs of conventional immunological methods enzyme-linked immunosorbent assay (ELISA) and fluorescent immunosorbent assay (FIA). The LOD of IPCR was more than 1 million times lower than that of ELISA, allowing the detection of 10 pg/L of ricin in human serum. IPCR appears to be the most sensitive method for the detection of ricin and other RIP (Lubelli *et al.*, 2006).

Haes *et al.* (2006) developed fluorescently tagged RNA aptamers, which bind ricin A-chain specifically and are then detected using capillary electrophoresis. It is able to detect sub-nanomolar concentrations (1 nM) of ricin.

A rapid and sensitive method to detect castor contamination, which determines DNA containing the ricin sequence, has been developed. The method is PCR based and makes use of a targeting primer sequence, Ricin-F4/R4 (He *et al.*, 2007).

Three recent publications describe high sensitivity methods for the detection of ricin in biological samples including human serum:

A rapid and sensitive method based on the glycosylase activity of ricinon ribosomal RNA detection of castor contamination in milk and liquid egg samples has been developed. It makes use of real-time polymerase chain reaction (PCR) using TaqMan and SYBR Green I dye. Primers against a highly conserved sequence from the 18S ribosomal RNA gene were used as a positive control for DNA extraction and PCR reaction efficiency. The quantity and quality of DNA prepared from castor spiked or non-spiked milk and egg samples obtained from three different DNA extraction methods were compared and the cetyl-trimethylammonium-bromide (CTAB) method yielded the highest quality of DNA and the most sensitive detection of castor DNA in both milk and liquid egg matrixes. Both real-time PCR systems detected 100 ng of stable acetone powder which was prepared from castor seeds, corresponding to 5 ng of ricin, in 1 mL of milk or liquid egg, which is well below the human toxic dose. The authors concluded that the real-time

PCR method for detection of intentional castor contamination can be applied to milk and egg matrixes (He *et al.*, 2007).

A very sensitive immuno polymerase chain reaction (IPCR) assay was developed for the detection of type I and type II RIPs that combines the specificity of immunological analysis with the exponential amplification of PCR. The limit of detection (LOD) of the technique was compared with the LODs of conventional immunological methods enzyme-linked immunosorbent assay (ELISA) and fluorescent immunosorbent assay (FIA). The LOD of IPCR was more than 1 million times lower than that of ELISA, allowing the detection of 10 pg/L of ricin in human serum. IPCR appears to be the most sensitive method for the detection of ricin and other RIP (Lubelli *et al.*, 2006).

Microscopical detection

In the control of feed materials for compliance with Directive 2002/32/EC[15], light microscopy is the principal means of detection used for botanical impurities. The successful identification of contaminants depends on several factors including the skill of the operator, the availability of reference material and the degree to which the sampled material has been processed. Commination and heat/pressure treatments can destroy much, or all, of the anatomical/histological feature on which identification is based. Hence this method is time consuming and less accurate than chemical analyses

3. Current legislation

Annex 1 to Directive 2002/32/EC contains a list of compounds that are undesirable in animal feed and their maximum levels in different feed commodities. The current EU maximum levels for ricin containing plants in feed materials are given in Table 1. It should be noted that for castor oil plants maximum level refers to plant husks, which can be detected by microscopic examination.

[15] OJ L 140, 30.5.2002, p. 10–22

Table 1. EU legislation on ricin containing plant materials used as feed.

Undesirable substances	**Product intended for animal feed**	**Maximum content in mg/kg relative to a feedingstuff with a moisture content of 12%**
Castor oil plant – *Ricinus communis* L.	All feedingstuffs	10 (expressed in terms of castor-oil plants husks)
Croton - *Croton tiglium* L.	All feedingstuffs	Seeds and fruit of the plant as well as their processed derivates may only be present in feedingstuffs in trace amounts not quantitatively determinable

4. Occurrence in feed materials

Ricin occurs only in the castor oil plant (*Ricinus communis L.*), where it is predominantly found in the seed[16]. Most outbreaks of poisoning in animals result from them being fed with improperly detoxified castor bean meal (Cooper and Johnson, 1984). Whilst the seeds are the primary source of toxin, the rest of the plant may also be considered to be slightly toxic. There have been reports of livestock being poisoned after consuming castor bean plants (e.g. Lewis *et al.*, 1995; Tokarnia *et al.*, 2002), but poisoning in livestock is rarely reported because the castor oil plant is seldom grazed by stock when other pasture plants are available. Clearly the risk of ingestion of the whole plant is greatest where it grows. Although it is most common in tropical countries, it is probably indigenous to some areas in the south eastern Mediterranean region (Phillips and Martyn, 1999).

In certain countries, castor oil is produced by *cold pressing* of castor beans without pre-heating the seeds. Ricin is not destroyed during cold pressing but ends up in the cake (or pomace). The ricin content in the pomace is typically about 5% or less (OPCW, 1994), and because of this content, de-oiled castor seed cake is usually used as a fertilizer (Kumar, 1992).

The extracted meal contains about 1% residual oil. Screw-pressed cake has about 6-8% residual oil and double-pressed cake 5-7%. A number of physical and chemical methods for detoxifying castor seed meal have been investigated, and have been reviewed by Anandan *et al.* (2004). Physical treatments included Soaking (3, 6 and 12 hours), steaming (30 and 60 min), boiling (30 and 60 min), autoclaving (15psi, 30 min; 15psi, 60 min) and heating (100°C 30 min; 120°C 25 min), while the chemical methods consisted of treatment with ammonia (7.5, 12.5 mL/kg of

[16] It is present in lesser concentrations in other parts of the plant, but these are not consumed by livestock.

castor cake), formaldehyde (5, 10 g/kg), lime (10, 20 and 40 g/kg), sodium chloride (5, 10 and 20 g/kg), tannic acid (5, 10 g/kg) and sodium hydroxide (2.5, 5 and 10 g/kg). Only autoclaving (15psi., 60 min) and lime treatment (40 g/kg) destroyed the toxin**.** Effective detoxification has been achieved by roasting at 140°C for 20 minutes (Okoroe *et al.*, 1985), or treatment with calcium hydroxide or sodium bicarbonate, or autoclaving (at 20 psi for 60 minutes; Rao *et al.*, 1988). Once detoxified, castor meal can be safely fed to livestock (Okoroe *et al.*, 1985; Rao *et al.*, 1988; Puttaraj, 1994).

For *Croton tiglium* the meal contains toxic components and is not used as a feed for livestock.

A call was launched by the EFSA for ricin occurrence data in feed materials within the Member States in preparation of this Opinion, but no data were received. In a survey of undesirable substances in feedingstuffs (van Raamsdonk, 2007), two animal health incidences – in 1996 and 1998 - were reported that were associated with *Ricin communis* in feed. No data has been identified on the occurrence of seeds of *Ricin communis*, *Croton tiglium* or *Jatropha curcas* as impurities in feed materials.

5. Estimating the intake by farm livestock

Detoxified castor seed meal has been used as a feed for livestock, but because of the presence of ricin and other toxins de-oiled castor seed cake is seldom used as a livestock feed (Aganga and Tshwenyane, 2003). No statistics are available on quantities of castor seed meal imported into the European Union or used as livestock feed. The castor bean plant is primarily a crop of tropical climates, and it is unlikely that any commercial plantings occur in the EU. Oil extraction takes place predominantly in the countries in which the seeds are produced, and thus little if any castor seed meal is produced in the EU (less than 5000 tonnes). With crude fibre contents typically in excess of 30% (Göhl, 1975), the nutritive value of castor oil seed cake is low, and therefore it is unlikely to be economic to import this material as an animal feed into the EU.

The CONTAM Panel is not aware of any feed producers using castor seed meal as a feed for livestock in the EU, and therefore with this caveat, exposure of animals would only be expected as a result of accidental contamination.

6. Adverse effects on livestock, pets and fish

Ricin is toxic to all animal species. Although there is wide variation in sensitivity to the toxin, both between and within species (Waller *et al.,* 1966), the lethal doses are small. The horse is the most susceptible animal, and the lethal oral dose of castor seed in horse is reported to vary from 7 to >300 mg/kg b.w. (Bornemann, 1992). The approximate lethal doses of castor seed in cows, sheep and pigs are 1-2 g/kg b.w., and reported lethal doses of castor seed in goats and hens are 5.5 and 14 g/kg b.w., respectively (Völker, 1950). Depending on the concentration of the ricin in feed, animals poisoned by eating feed containing ricin may show symptoms within a few hours, or up to three days later (Cooper and Johnson, 1984). For those that do not die, recovery may take several weeks as a result of severe tissue damage (Lensch, 1996). Inclusion of up to 5% detoxified castor bean meal in the diet has not been shown to cause adverse effects or nutritive problems in lactating dairy cows, beef cattle, sheep (Bris and Algeo, 1970; Purushotham *et al.,* 1986; Rao *et al.,* 1988). Poultry may tolerate higher inclusion rates of detoxified castor pomace (Okamoto *et al.,* 1965)[17].

6.1. Ruminants

Detoxified castor bean meal has been fed to lactating dairy cows over a 14-month period without signs of adverse effects (Robb *et al.,* 1974). Diets containing 10 and 20% castor bean meal, with or without 0.5% added castor oil, were compared with control diets containing 10% cottonseed meal and 0.5% cottonseed oil. No abnormal production or fertility conditions related to the castor bean meal were noted in the cows. The concentration of ricin in the detoxified castor bean meal was 11.6 mg/kg, reported by the authors to be about 5000 times less than in raw castor seed. Thus, the ricin concentration in the total feed was 1.2-2.4 mg/kg, which may correspond to approximately 0.04-0.08 mg/kg b.w. per day. Ricin was not detected in the milk.

Marion (1967) fed six young heifers (mean weight 138 kg) with castor bean meal maintained to contain at least 3.6 g/kg of ricin (determined by a mouse bioassay). The first 2 weeks each animal received 0.090 kg castor bean meal per day (at least 2.3 mg ricin/kg b.w. per day). Thereafter, the animals were divided into two groups of three animals. One group received 0.18 kg castor bean meal while the other group continued on the previous ration for the next two weeks. Then the levels of castor bean meal were increased weekly up to a level after 9 weeks of 0.91 kg per day for the high ration group and 0.45 kg in the low ration group. These levels were maintained for

[17] In some reports, concentrations of ricin in detoxified meal have not been given. Where levels are stated, these are given in this Opinion.

another 5 weeks, before another increment within a two-week period up to 1.36 kg in the high ration group and 0.68 kg in the low ration group. These levels were fed the last 12 weeks of the 196-day trial. The highest ration level of 1.36 kg castor bean meal constituted approximately 20% of the animals total ration (corresponds to 4.1-5.5 g/kg b.w. of castor bean meal per day, and a ricin dose of at least 15-20 mg/kg b.w. per day). There were no adverse clinical effects related to feeding of the castor bean meal, and the weight gain was satisfactory. Pathological examinations gave also negative results.

Outbreaks of ricin poisoning in cattle fed rations contaminated with castor bean husks have been described (Anderson, 1948; Geary, 1950; Fox, 1961). In dairy cows a fall in milk yield is typical as well as depression, inappetence and severe diarrhoea. The severity of the symptoms and the speed at which they appeared is related to dose. The poisoning often ends fatally and those that survive may need weeks for recovery. At autopsy intense inflammation of abomasum (the stomach) and intestines with corrosion of the mucous membranes, and petechiae of the heart were found. The minimum fatal dose of castor bean husks was about 0.5 g/kg b.w. The ricin concentration was not determined in these cases.

Adult sheep were fed 250 g of concentrate, which contained 15 or 30% castor bean meal containing 2.2 g ricin per kg (replaced groundnut cake), for 8 months (Rao *et al.*, 1984; Purushotham *et al*., 1985a). Thus, the animals were fed 38 or 75 g castor bean meal per day, corresponding to a daily ricin dose of 83 or 165 mg/animal per day. The body weights of the animals were not given, but if estimated to be 60 kg, these figures correspond to 0.6 and 1.3 g/kg b.w. per day of castor bean meal, and 1.4 and 2.8 mg/kg b.w. per day of ricin. The animals maintained their body weights during the entire experimental period and no signs of ill health due to ricin were observed. However, the colour of urine and faeces was dark brown in groups fed castor bean meal. At autopsy the mucous membrane of the intestines was congested and a thick, mucous exudate was seen within the lumen in most of the ricin exposed animals. The kidneys were congested with frequent haemorrhages. By microscopy, a dose related acute tubular necrosis was observed in kidneys in particular, but also in the liver, intestine, spleen and lymph nodes. Biochemical blood parameters did not show significant effects.

Adult sheep were fed 250 g of a concentrate which contained 30% of either undetoxified or detoxified (by heating) castor bean meal or groundnut cake for 8 months for haematological studies (Purushotham *et al.,* 1985b). The concentration of ricin in the undetoxified castor bean meal was 2.2 g/kg, giving a daily dose of 165 mg ricin/animal per day, corresponding to approximately 2.8 mg/kg b.w. per day (assuming a body weight of 60 kg). Significantly increased numbers of lymphocytes and reduced numbers of neutrophile leucocytes were found in animals fed the castor bean meal with ricin in particular, but a similar pattern was also found in

animals fed the detoxified castor bean meal when compared with those fed the groundnut cake ration.

A sheep flock intoxicated with castor beans in miscellaneous garden waste (Aslani *et al.*, 2007). Forty-five animals showed clinical symptoms assumed to be caused by castor beans and 17 died. The clinical signs included weakness, salivation, profuse watery diarrhoea, dehydration, mydriasis, teeth grinding, hypothermia and recumbency. The heamatocrit, serum BUN, creatinine and phosphorus were increased, and there were high activities of serum CK and AST. Autopsies revealed severe gastroenteritis, cardiac haemorrhage and necrosis, hepatic necrosis and acute tubular necrosis in the kidneys.

To conclude on ruminants, if acclimatised with moderate doses, cattle seem to tolerate relatively high levels of ricin for long time exposure. Thus, approximately 15-20 mg/kg b.w. per day of ricin corresponding to approximately 4.1-5.5 g/kg b.w. per day of castor bean meal was well tolerated in acclimatised heifers. In sheep, approximately 1.4 mg/kg b.w. per day of ricin corresponding to 0.6 g/kg b.w. per day of castor bean meal caused morphological changes in several tissues.

6.2 Horses

Bornemann (1992) reported that of three horses given a single dose of 2.5 g castor seeds one got colic and died (7 mg/kg b.w.) while the other two showed incidentally reduced feed intake (7 and 8 mg/kg b.w.). The fatal dose is very variable as the same author also reported that only two of seven horses dosed with 100-240 mg castor seeds per kg b.w. died, as well as one of two horses dosed with 300 mg castor seeds per kg b.w. However severe symptoms were observed in most of these horses. A detailed description of the appearance of horses at all stages of castor seed intoxication has been given by McCunn *et al.* (1945). In the early stages animals were dull. Later they showed signs of incoordination, profuse watery diarrhoea, and in severe cases sweating, tetanic spasms of the muscles, tachycardia and arrhythmia.

6.3. Pigs

Pigs poisoned from a mash made of meal contaminated with castor bean husk, vomited, had diarrhoea, were weak, showed some incoordination and signs of abdominal pain (Geary, 1950). The level of castor bean husk was about 12 g/kg meal, corresponding to a dose of approximately 0.5 g/kg b.w. of castor bean husk.

6.4. Poultry

Growing chicks (150 days old) were fed non-roasted castor beans at 10% of the diet, or roasted castor beans at 10, 15 or 20% of the diet for 6 weeks (Okorie and Anugwa, 1987). Roasting was performed to destroy ricin, but the ricin content of the diets was not determined. Feed intake, growth rate, feed conversion ratios and mortalities were monitored and compared with an iso-nitrogenous and iso-caloric control group without castor beans. Significantly reduced feed intake and weight gain, and increased feed conversion ratio were found at all diets with castor beans. The most dramatic effects were found in birds fed the non-roasted castor beans, and 83% of the birds died during the experimental period. The effects on birds fed the roasted beans were related to the castor bean concentration. The mortality was not significantly increased for birds fed the roasted beans. The body weights of the birds were not given but if it is assumed that the feed intake related to body weight was approximately 10% the daily dose of castor beans was approximately 10 g/kg b.w. for birds fed the diet with 10% castor beans.

Pullets poisoned from a mash made of meal contaminated with castor bean husk, showed dullness, drooping wings, ruffled feathers and greyish-coloured wattles and combs (Geary, 1950). The level of castor bean husk was about 12 g/kg meal, corresponding to a single dose of approximately 1 g/kg b.w.

Jensen and Allen (1981) describe an outbreak of castor bean poisoning which killed several thousands ducks in USA during 1969-71. The clinical signs resembled those of botulism, except for mucoid, blood-tinged excreta. The commonest lesions were severe fatty change in the liver, widely distributed internal petechial haemorrhages or eccymoses, and catarrhal enteritis. Experimental administration of castor beans in mallard duck indicated a LD_{50} of 3-4 beans (0.8-1.3 g) per bird, corresponding to 0.7-1.2 g castor beans per kg b.w.

Up to 40% detoxified castor bean meal has been used in chick rations (Vilhjalmsdottir and Fisher, 1971). In this trial, boiling proved to be an effective toxin removal method. Feeding hot-water extracted castor bean meal, together with appropriate amino acid supplementation, produced gains that were comparable to those achieved using soybean meal.

To conclude on birds, approximately 10 g/kg b.w. per day of castor beans reduced feed intake, weight gain and led to high mortality in growing chicks. A single dose of approximately 1 g/kg b.w of castor bean husk produced incidentally poisoning of pullets. In mallard ducks lethal dose is approximately 0.7-1.2 g castor beans per kg b.w.

6.5. Rabbits

No data on oral exposure are available.

6.6. Dogs

Injected ricin has a very low LD_{50} in dogs (1-1.75 µg/kg b.w.) (Fodstad *et al.*, 1979). Albretsen *et al.* (2000) evaluated cases of castor bean intoxications in dogs reported to the National Poison Control Center in USA during January 1987 to December 1998. In total 98 cases were reported during the 12-year period. The most commonly reported clinical signs were vomiting, depression, and diarrhoea. Signs developed most frequently within six hours following ingestion (range 0.5 to 42 hours). Death or euthanasia occurred in 9% of the cases. The severity of clinical signs following castor bean ingestion may depend on whether the beans were chewed or swallowed whole. Insufficient data were available to estimate a lethal dose. However, the ingestion of just one castor bean was sufficient to cause clinical signs in some dogs.

Cases of ricin intoxications in dogs (doses not given) have also recently been described by others (Soto-Blanco *et al.*, 2002; Stein *et al.*, 2006; Mouser *et al.*, 2007).

An organic fertilizer containing 25% extracted castor seed caused intoxication in dogs (Kriger-Huber, 1980). The author reported on 5 cases of intoxications whereof 3 were lethal.

6.7. Fish

No data on adverse effects in fish were identified.

7. Toxicokinetics

Toxicokinetic data on ricin are very limited. Ricin has been administered orally in rats as 10 mg/kg b.w. of ricin D (corresponding to 1/3 LD_{50}) and its distribution in the gastrointestinal tract, body fluids and principal organs was determined by an enzyme immunoassay. The immunoreactive ricin was identified by gel filtration followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, protein blotting and immunobinding. 75% of ricin was found in the stomach and small intestine within 2 hours, and then most of it was transferred to the large intestine after 24 hours. An *in vitro* toxicity test of immunoreactive ricin in the blood and lymph from treated rats showed that ricin was absorbed from the small intestine into the tissues and organs via the circulatory systems (lymphatic and blood vessels) as the active ricin. The absorption of ricin from the gastrointestinal tract was mediated through the blood rather than the lymphatic system. Ricin, after absorption, was detected in liver and spleen and ricin found in the liver was predominantly in the form of intact ricin, although an undetectable amount of ricin in other organs cannot be excluded. From these results it can be inferred that a small fraction of

orally-given ricin was transferred to the circulatory system. The rat died from the oral ricin treatment (Ishiquro *et al.,* 1992). The lectin activity of ricin plays an important role in the absorption of ricin from the small intestine. The absorption of ricin protein is further enhanced by its high toxicity on intestinal mucosa (Ishiquro *et al.,* 1992).

Ricin was recently measured in tissues using ELISA (sensitivity limit approximately 200 pg/mL) in conjunction with pure ricin standards after inhalation and oral dosing in rats. Extracts from tissues sampled, including lung, blood, liver and spleen, tested positive for ricin with the maximum yield in lung associated fractions for pulmonary dosing and liver tissue for oral administration. Time course analysis at 24 and 48 hours indicated the progression of ricin from surfaces of the lung into the lung tissue. Inter-subject variations were observed in the case of oral dosing (Cook *et al.*, 2006).

8. Carry-over and residues

Jaffé (1969) suggested that ricin might be present in the milk of lactating guinea pigs that had been injected with the agglutinin after the birth of the litters because the suckling young became markedly resistant against subsequent injections of the toxins. However, there is no clear evidence that ricin consumed by livestock is transferred to livestock products (milk, meat or eggs). Robb *et al.* (1974) reported a study in which diets containing 10 and 20% detoxified castor bean meal were fed to lactating dairy cows over a 14-month period. No abnormal effects on production or reproduction were noted, nor was there any apparent transfer to milk of ricin. Calves and rats fed milk from test cows showed neither apparent muscle residue accumulation nor abnormalities of internal organs.

9. Human dietary exposure

Except for exposures to ricin via the accidental intake of castor beans (*ricinus communis*) human exposure via food is unlikely to occur.

CONCLUSIONS

Chemistry and occurrence in plants and parts thereof

- Ricin is a toxic glycoprotein, with several minor variants, which consist of two polypeptide chains each of approximately 30 kDa and joined by a disulfide bond. Ricin is a type II ribosomal inhibitory protein (RIP II).
- Ricin is found in the seeds (beans) (and also to some extent in the husks) of the castor oil plant (*Ricinus communis L.*), a member of the Spurge family of plants (Euphorbiaceae). A limited number of other plants in the same family contain type II RIPs, i.a. subtropical leguminous climber *Abrus precatorius* L. and, *Croton tiglium* L. which contain abrin and crotin I, respectively.
- *Croton tiglium* L. (Euphorbiaceae) has long been known as a toxic plant. The seeds are particularly toxic, containing a number of skin-irritating phorbol esters and showing a strong laxative effect. The seeds also contain crotin I, which is a RIP II protein with a molecular mass of around 40 kDa. The seeds of *Croton tiglium* contain a number of toxins, which make it unsuitable as a feed for livestock.
- In the Terms of Reference, the plant *Jatropha curcas* was also requested to be considered, however, it does not contain a RIP II protein similar to ricin in *Ricinus communis* and crotin I in *Croton tiglium*. The toxicity of its seeds can be ascribed to the oil, which contain phorbol esters and this plant is therefore not relevant for this opinion on ricin. At present the available information on the toxic principles of *Jatropha curcas* is very limited and the Panel decided that a conclusive evaluation of these scarce data is not possible.

General toxicological effects

- Following uptake into cells by endocytosis ricin causes acute cell death by inactivation of ribosomal RNA, inhibiting protein synthesis. Because of its destruction in the intestinal tract, ricin is approximately 1000-fold more toxic following parenteral administration or inhalation, than by the oral route. Oral LD_{50} values in rats and mice were 20 to 30 mg/kg b.w., and the corresponding intra peritoneal LD_{50} value for mice is 22 µg/kg b.w. There are no data on chronic or reproductive toxicity, or genotoxicity of ricin.
- There are very few data available on the toxicity of crotin I, the only LD_{50} value for crotin I is 20 mg/kg b.w. after *intra peritoneal (i.p.)* administration in mice.

Adverse effects of ricin in target animals

- If accustomed to moderate doses of ricin, cattle seem to tolerate relatively high levels of ricin in feed for long time exposure. In accustomed heifers, 20 mg/kg b.w. per day of ricin (corresponding to approximately 5.5 g/kg b.w. per day of castor bean meal) was well tolerated. In sheep, approximately 1.4 mg/kg b.w. per day of ricin (corresponding to 0.6 g/kg b.w. per day of castor bean meal) caused morphological changes in several tissues.
- Acute ricin poisonings have been reported in horses. A single dose of 2.5 g castor seeds (7-8 mg ricin /kg b.w.) caused reduced feed intake, colic and death in horses. Higher levels have been tolerated in other studies.
- Pigs poisoned from a mash made of meal contaminated with 12 g husk/kg meal (corresponding to 0.5 g/kg b.w.), exhibited vomiting, diarrhoea, weakness, some in-coordination and signs of abdominal pain.
- In birds, approximately 10 g/kg b.w. per day of castor beans reduced feed intake, weight gain and led to high mortality in growing chicks. Pullets were poisoned by about 1 g/kg b.w. of castor bean husk. In mallard ducks, approximately 0.7-1.2 g castor beans/kg b.w. was lethal.

Ricin in feed materials

- The CONTAM Panel is not aware of any feed producers using castor seed meal as a feed for livestock in the EU. However, exposure of animals can not be excluded if castor oil seeds and other plants containing RIP II proteins are present as botanical impurities in feed materials.
- Following extraction of castor oil, ricin is left in the press-cake/castor bean meal. Castor oil production mainly takes place in countries outside the EU and only small quantities of castor bean meal (<5,000 tonnes) are produced in the EU. There are no data on the extent of the use of meal resulting from EU production or importation regarding its use as a feed or fertilizer.

Fate in animals and carry-over

- There are limited data on toxicokinetics and carry-over of ricin to products of animal origin (milk, meat or eggs). Livestock has a very low tolerance to ricin exposure via feed before clinical symptoms of toxicity will be manifest. It is therefore unlikely that highly exposed

animals would enter the food chain, and the CONTAM Panel considers the risk of ricin transfer to livestock products to be negligible.

Human exposure

- Except for exposures to ricin via the accidental intake of castor beans (*ricinus communis*) human exposure via food, under normal circumstances, is unlikely to occur.

RECOMMENDATIONS

- More information is needed on the occurrence of RIP II protein containing seeds as botanical impurities in feed materials.
- More information is needed on the occurrence of seeds *Ricinus communis*, *Croton tiglium* and *Jatropha curcas* as botanical impurities in feed materials.

REFERENCES

Adam, S.E.I. and Magzoub, M. 1975. Toxicity of Jatropha curcas for goats. Toxicology 4: 347-354.

Adolf, W., Opferkuch, H.J. and Hecker, E. 1984. Irritant phobol derivatives from four Jatropha species. Phytochemistry 23: 129-132.

Aganga, A.A. and Tshwenyane, S.O. 2003. Feeding Values and Anti - Nutritive Factors of Forage Tree Legumes. Pakistan Journal of Nutrition 2: 170-177

Ahmed, O.M.M. and Adam, S.E.I. 1979a. Effects of Jatropha curcas on calves. Vet. Pathol. 16: 476-482.

Ahmed, O.M.M. and Adam, S.E.I. 1979b. Toxicity of Jatropha curcas in sheep and goats. Res. Vet. Sci. 27: 89-96.

Albretsen, J.C., Gwaltney-Brant, S.M. and Khan, S.A. 2000. Evaluation of castor bean toxicosis in dogs: 98 cases. J. Am. Anim. Hosp. Ass., 36: 229-233.

Anandan, S., Kumar, G., Ghosh, J. and Ramachandra, K. 2004. Effect of different physical and chemical treatments on detoxification of ricin in castor cake**.** Animal Feed Science and Technology 120: 159-168.

Anderson, T.S. 1948. Castor poisoning in Ayrshire cattle. Vet. Rec. 60 (3): 28.

Anonymous, 2006. Analysing the Indian Stock Market - Castor and its derivatives. http://www.crnindia.com/commodity/castor.html

Aslani, M.R., Maleki, M., Mohri, M., Sharifi, K., Najjar-Nezhad, V. and Afshari, E. 2007. Castor bean (*Ricinus communis*) toxicosis in a sheep flock. Toxicon 49 (3): 400-406.

Audi, J., Belson, M., Patel, M., Schier, J. and Osterloh, J. 2005. Ricin poisoning – a comprehensive review. JAMA 294: 2342-2351.

Bagaria, A., Surendranath, K., Ramagopal, U.A., Ramakumar, S. and Karande, A.A. 2006. Structure-function analysis and insights into the reduced toxicity of Abrus precatorius Agglutinin 1 in relation to Abrin. J. Biol. Chem. 281: 34465-34474.

Balint, G.A. (1974). Ricin: the toxic protein of castor beans. Toxicology 2, 77-102.

Barbieri, L., Battelli, M. and Stirpe, F. 1993. Ribosome - inactivating protein from plants. Biochem Biophys Acta 1154: 237-282.

Becker, K. and Makkar, H.P.S. 1998. Effects of phorbol esters in carp (Cyprinus carpio L.). Vet. Hum. Toxicol. 40: 82-86.

Benge, M. 2006. Assessment of the potential of Jatropha curcas, (biodiesel tree,) for energy production and other uses in developing countries. Posted on ECHO's website July 2006 and updated August 2006.

Bigalke, H. and Rummel, A. 2005. Medical aspects of toxin weapons. Toxicology 214: 210-220.

Bismuth, C., Borron, S.W., Baud, F.J. and Barriot, P. 2004. Chemical weapons: documented use and compounds on the horizon. Toxicol.Lett. 149 : 11-18.

Bornemann. 1992. Über die tödliche Dosis des semen Rizini bei Pferden. Deutsche Tierärzt. Woch. 30 (10): 123-127.

Bradberry, S.M., Dickers, K.J., Rice, P., Griffiths, G.D. and Vale, J.A. 2003. Ricin poisoning. Toxicol.Rev. 22: 65-70.

Bris, E.J. and Algeo, J.W. 1970. Castor bean byproducts for cattle rations. Feedstuffs, 42(20): 26-28.

Buchheister, G.A. and Ottersbach, G. 1919. Handbuch der drogisten-praxis. Ein Lehr- und Nachschlagebuch für drogisten, farbwarenhändler usw. Berlin: Verlag von Julius Springer, pp. 333 and 513.

Chen, M.-H., Zhou, K.-J., Fu, Z.-J. and Pan, K.-Z. 1993. Preliminary crystallographic studies of crotin II. J. Mol. Biol. 234: 908-909.

Chen, M.-H., Ye, X.-M. and Wang, Z.R. 2003. Crotins, plant toxins from the seed of Croton tiglium (Congress abstract). Toxicology 191 (1): 48.

Cmiech, H.A., Morley, M. and Gee, D.J. 1985. The immunocytochemical detection of ricin I. Preliminary immunofluorescence studies. Histochem. J. 17: 859-866.

Cook, D.L., David, J., and Griffiths, G.D. 2006. Retrospective identification of ricin in animal tissues following administration by pulmonary and oral routes. Toxicology 223: 61-70.

Cooper, M.R. and Johnson, A.W. 1984. Poisonous Plants in Britain, and their effects on Animals and Man. Ministry of Agriculture, Fisheries and Food Reference Book 161. Her Majesty's Stationary Office, London

Cosmetic Ingredient Review Expert Panel, 2007. Final Report on the Safety Assessment of Risinus Communis (Castor) seed Oil, Hydrogenated Castor Oil, Glyceryl Ricinoleate, Glyceryl Ricinoleate SE, Ricinoleic Acid, Potassium Ricinoleate, Sodium Ricinoleate, Zink Ricinoleate, Cetyl Ricinoleate, Ethyl Ricinoleate, Glycol Ricinoleate, Isopropyl Ricinoleate, Methyl Ricinoleate, and Octyldodecyl Ricinoleate. International Journal of Toxicology 26(suppl. 3): 31-77.

Darby, S.M., Miller, M.L. and Allen, R.O. 2001. Forensic determination of ricin and the alkaloid marker ricinine from catsor castor bean extracts. J. Forensic Sciences 46: 1033-1042.

Dertzbaugh, M.T., Rossi, C.A., Paddle, B.M., Hale, M., Poretski, M. and Alderston, M.R. 2005. Monoclonal antibodies to ricin: In vitro inhibition of toxicity and utility as diagnostic reagents. Hybridoma 24(5): 236-243.

Dill, K., Montgomery, D.D., Ghindilis, A.L., Schwarzkopf, K.R., Ragsdale, S.R. and Oleinikov, A.V. 2004. Immunoassays based on electrochemical detection using microelectrode arrays. Biosensors and bioelectroniscs 20: 736-742.

Doan, L.G. 2004. Ricin: mechanism of toxicity, clinical manifestations, and vaccine development. A review. J.Toxicol.Clin.Toxicol. 42: 201-208.

El Badwi, S.M.A., Mousa, H.M., Adam, S.E.I. and Hapke, H.J. 1992. Response of Brown Hissex chicks to low levels of Jatropha curcas, Ricinus communis or their mixture. Vet. Hum. Toxicol. 34: 304-306.

El Badwi, S.M., Adam, S.E.I. and Hapke, H.J. 1995. Comparative Toxicity of Ricinus Communis and Jatropha Curcas in Brown Hisex Chicks. Deutsche Tierarztliche Wochenschrift 102: 75-77.

El-Nikhely, N., Helmy, M., Saeed, H.M., Shama, L.A.A. and El-Rahman, Z.A. 2007. Ricin A chain from Ricinus sanguineus: DNA Sequence, structure and toxicity. Prot. J. 26: 481-489.

Endo, Y. and Tsurugi, K. 1987. RNA N-glycosidase activity of ricin A-chain. Mechanism of action of the toxic lectin ricin on eukaryotic ribosomes . J. Biol Chem. 262: 8128-8130.

Endo, Y., Mitsui, K., Motiguki, M. and Tsurugi, K. 1987. The mechanisms of ricin and related toxins on eukaryotic ribosomes. J. Biol Chem. 262: 5908-5912.

Fodstad, O., Johannessen, J.V., Schjerven, L. and Pihl, A. 1979. Toxicity of abrin and ricin in mice and dogs. J Toxicol Environ Health. 5: 1073-1084.

Fox, M.W. 1961. Castor seed residue poisoning in dairy cattle. Vet Rec 73(36): 885-886.
Frederiksson, S-Å, Hulst, A.G., Artursson, E., de Jong, Ad. L., Nilsson, C. and van Baar, B.L.M. 2005. Forensic Identification of Neat Ricin and of Ricin from crude Castor Bean Extracts by Mass Spectrometry. Anal. Chem. 7: 1545-1555.
Frenoy, J.P. 1986. Effects of physical environment on the conformation of ricin. Biochem. J. 240: 221–226.
Frigerio, L., Jolliffe, N.A., Di Cola, A., Hernandez, F.D., Paris, N., Neuhaus, J.-M., Lord, J.M., Ceriotti, A. and Roberts, L.M. 2001. The internal propeptide of the ricin precursor carries a sequence-specific determinant for vacuolar sorting. Plant Physiol. 126: 167-175.
Frigerio, L., Vitale, A., Lord, J.M., Ceriotti, A., and Roberts, L.M. 1998. Free ricinA chain, proricin, and native toxin have different cellular fates when expressed in tobaccor protoplasts. J. Biol. Chem. 273: 14194-14199.
Gaigalas, A.K., Cole, K.D., Bykadi, S. Wang, L., DeRose, P. 2007. Photophysical Properties of Ricin. Photochemistry and Photobiology 83 (5): 1149–1156.
Geary, T. 1950. Castor bean poisoning. Vet. Rec. 62 (32): 472-473.
Ghandi, V. M., Cherian, K. M., and Mulky, M. J. 1995. Toxicological studies on Ratanjyot oil. Food Chem. Toxicol. 33: 39-42.
Göhl, B. 1975. Tropical Feeds. FAO. Rome, pp 285–453.
Goldman, E.R., Clapp, A.R., Anderson, G.P., Uyeda, H.T., Mauro, J.M., Medintz, I.L. and Mattoussi, H. 2004. Multiplexed toxin analysis using four colors of quantum dot fluororeagents. Anal. Chem. 76: 684-688.
Griffiths, G.D., Newman, H. and Gee, D.J. 1986. Identification and quantification of ricin toxin in animal tissues using elisa. J. Forensic Sci. Soc. 26: 349-358.
Haes, A.J., Giordano, B.C., and Collins, G.E. 2006. Aptamer-based detection and quantitative analysis of ricin using affinity probe capillary electrophoresis. Anal.Chem. 78: 3758-3764.
Hänsel, R. and Haas, H. 1983. Rizinusöl, In “Therapie mit phytopharmaka”, Berlin: Springer-Verlag, pp. 167-168.
He, X., Carter, J.M., Brandon, D.L., Cheng, L.W., and McKeon, T.A. 2007. Application of a real time polymerase chain reaction method to detect castor toxin contamination in fluid milk and eggs. J. Agric.Food Chem. 55: 6897-6902.
Hecker, E. 1968. Cocarcinogenic principles from the seed oil of Croton tiglium and from other Euphorbiaceae. Cancer Research 28: 2338-2349.
Heller, J. 1996. Physic nut Jatropha Curcas L. Promoting the conservation and use of underutilized and neglected crops; Institute of Plant Genetics and Crop Plant Research (Gartersleben) and International Plant Genetic Resources Institute: Rome, 1996; Vol. 1.
Hines, H.B. and Brueggemann, E.E. 1994. Factors affecting the capillary electrophoreis of ricin, a toxic glycoprotein. J. Chriomatogr. A 670: 199-208.
http://www.echotech.org/mambo/index.php?option=com_docman&task=doc_download&gid=178
Ishiguro, M., Tanabe, S., Matori, Y. and Sakakibara, R. 1992. Biochemical studies on oral toxicity of ricin. IV. A fate of orally administered ricin in rats. J. Pharmacobiodyn. 15: 147-156.
Jaffé, W.G. 1969. Hemagglutinins. In Toxic Constituents of Plant Foodstuffs (Eds. I.E. Liener). Acadamic Press, New York, pp 69-102.

Jensen, W.I. and Allen, J.P. 1981. Naturally occurring and experimentally induced castor bean (Ricinus communis) poisoning in ducks. Avian Dis., 25: 184-194.

Johnson, R.C., Lemire, S.W., Woolfitt, A.R., Ospina, M., Preston, K.P., Olson, C.T. and Barr, J.R. 2005. Quantification of ricinine in rat and human urine: a biomarker for ricin exposure. Journal of Analytical Toxicology. 29(3): 149-155.

Katzin, B.J., Collins, E. J. and Robertus, J.D. 1991. The structure of ricin A chain at 2.5 A. Proteins 10: 251-259.

Kim, S.K., Hancock, D.K., Wang, L., Cole, K.D. and Reddy, P.T. 2006. Methods to characterize ricin for the development of reference materials for testing detection devices. J. Res. Natl Inst. Stand. Technol. 111: 313–324.

Kingsbury, J.M. 1964. Poisonous plants of the United States and Canada; Prentice-Hall Inc.: Englewood Cliffs, NJ.

Koja, N., Shibata, T. and Mochida, K. 1980. Enzyme-linked immunoassay of ricin. Toxicon 18: 611-618.

Kozlov, J.V., Sudarkina, O.J. and Kurmanova, A.G. 2006. Ribosome-inctivating lectins of plants. Mol. Biol. 40: 635-646.

Krieger-Huber, S. 1980. Rizin-Vergiftungen mit tödlichem Ausgang bei Hunden nach Aufnahme des biologischen Naturdüngers ”Oscorna animalin”. Kleintier-Praxis 25: 281-286.

Kulkarni, M.L., Sreekar, H., Keshavamurthy, K.S. and Shenoy, N. 2005. Jatropha curcas – Poisoning. Indian Journal of Pediatrics 72: 75-76.

Kumar, R. 1992. Anti-nutritional factors, the potential risks of toxicity and methods to alleviate them. In Legume trees and other fodder trees as protein sources for livestock. Proceedings of the FAO Expert Consultation, October 1991 (Eds: A. Speedy and P.-L. Pugliese). http://www.fao.org/DOCREP/003/T0632E/T0632E10.htm

Kumar, O., Lakshmana Rao, P.V., Pradhan, S., Jayaraj, R., Bhaskar, A.S., Nashikkar, A.B. and Vijayaraghavan, R. 2007. Dose dependent effect of ricin on DNA damage and antioxidant enzymes in mice. Cell Mol.Biol. (Noisy.-le-grand) 53: 92-102.

Kumar, O., Sugendran, K. and Vijayaraghavan, R. 2003. Oxidative stress associated hepatic and renal toxicity induced by ricin in mice. Toxicon 41: 333-338.

Lamb, F.I., Roberts, L.M. and Lord, J.M. 1985. Nucleotide sequence of cloned cDNA coding for preproricin. Eur. J. Biochem. 148: 265-270.

Leith, A.G., Griffiths, G.D. and Green, M.A. 1988. Quantification of ricin toxin using a highly sensitive avidin/biotin enzyme-linked immunosorbent assay. J. Forensic Sci. Soc. 28: 227-236.

Lensch, J. 1996. [Ricin poisoning in cattle.]. In German. Tierärztliche Praxis 21: 21-22.

Lewis, L.D., Knight, A., Lewis, B. and Lewis, C. 1995. Equine Clinical Nutrition: Feeding and Care. Blackwell Publishing

Liberalino, A.A.A., Bambirra, E.A., Moraes-Santos, T. and Viera, E.C. 1988. Jatropha curcas L. seeds: Chemical analysis and toxicity. Arq. Biol. Technol. 31: 539-550.

Lin, J., Yan, F., Tang, L. and Chen, F. 2003. Antitumor effects of curcin from seeds of Jatropha curcas. Acta Pharmacol Sin. 24: 241-246.

Lin, T.T.-S. and Li, S.S.-L. 1980. Purification and physiochemical properties of ricins and agglutinins from Ricinus communis. Eur. J. Biochem. 105: 453-459.

Lock, J.M. 1989. Legumes of Africa, a check-list. Kew: Royal Botanic Gardens, pp. 100.

Lord, J. M., Roberts, L.M. and Robertus, J.D. 1994. Ricin: structure, mode of action and some current applications. FASEB Journal 8 : 201-208.

Lubelli, C., Chatgilialoglu, A., Bolognesi, A., Strocchi, P., Colombatti, M. and Stirpe, F. 2006. Detection of ricin and other ribosome-inactivating proteins by an immuno-polymerase chain reaction assay. Anal.Biochem. 355: 102-109.

Makkar, H.P.S. and Becker, K. 1997. Jatropha curcas toxicity: Identification of toxic principle(s). Proceedings 5thInternational Symposium on Poisonous Plants, San Angelo, Texas, May 19-23.

Makkar, H.P.S., Becker, K., Sporer, F. and Wink, M. 1997. Studies on nutritive potential and toxic constituents of different provenances of Jatropha curcas. J. Agric. Food Chem. 45: 3152-3157.

Marion, P.T. 1967. Castor meal - a new protein supplement. Proceedings, Western Section, American Society of Animal Science, 18: 153-157.

Martinez-Herera, J., Siddhuraju, P., Francis, G., Davila-Ortiz, G. Anmd Becker, K. 2006. Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of Jatropha curcas L. from Mexico. Food Chemistry 96: 80-89.

McCunn, J., Andrews, H. and Clough, G.W. 1945. Castor-bean poisoning in horses. Vet. J., 101: 136-138.

Montfort, W., Villafranca, J.E., Monzingo, A.F., Ernst, S., Katzin, B., Rutenber, E., Xuong, N. H., Hamlin, R. and Robertus, J.D. 1987. The three dimentional structure of ricin at 2.8A. J. Biol. Chem. 262: 5398-5403.

Mouser, P., Filigenzi, M.S., Puschner, B., Johnson, V., Miller, M.A. and Hooser, S.B. 2007. Fatal ricin toxicosis in a puppy confirmed by liquid chromatography/mass spectrometry when using ricinine as a marker. J. Vet Diagn. Invest. 19: 216-220.

Muldoon, D.F., Hassoun, E.A. and Stohs, S.J. 1992. Ricin-induced hepatic lipid peroxidation, glutathione depletion, and DNA single-strand breaks in mice. Toxicon. 30(9): 977-84.

Muldoon, D.F., Bagchi, D., Hassoun, E.A. and Stohs, S.J. 1994. The modulating effects of tumor necrosis factor alpha antibody on ricin-induced oxidative stress in mice. J Biochem Toxicol. 9(6): 311-8

Muldoon, D.F., Hassoun, E.A. and Stohs, S.J. 1996. Role of iron in ricin-induced lipid peroxidation and superoxide production. Res Commun Mol Pathol Pharmacol. 92(1): 107-18.

Okamoto, S., Koga, O., Goto, I., Aramaki, T., Funatsu, M., Takahashi, T., Yumaguchi, C., Kusakawa, S. and Murase, K. 1965. Studies of utilization of castor pomace for animal feed. III: Further studies on detoxification and availability as a poultry feed. Jap. Poult. Sci. 2(1): 1-10 (English summary Biol. Abstr. 47, No. 22893-1966).

Okorie, A.U. and Anugwa, F.O.I. 1987. The feeding value of roasted castor oil bean (*Ricinus communis*) to growing chicks. Plant Foods Hum. Nutr. 37: 97-102.

Okorie, A.U., Anugwa, F.O.I., Anamelechi ,G.C. and Nwaiwu, J. 1985. Heat-treated castor oil bean (Ricinus communis): a potential livestock protein-supplement in the tropics. Nutrition Reports-International 32(3): 659-666.

Olsnes, S. 2004. The history of ricin, abrin and related toxins. Toxicon 44: 361-370.

Olsnes, S., Wesche, J. and Falsnes, P.O. 1999. Binding, uptake, routing and translocation of toxins with intracellular sites of action. In: Alouf, J.E., Freer, J.H. (Eds.), The Comprehensive Sourcebook of Bacterial Toxins, Academic Press, London, pp. 73-93.

OPCW (Organisation for the Prohibition of Chemical Weapons), 1994. Preparatory Commission for the Organisation for the Prohibition of Chemical Weapons http://www.opcw.org/docs/pseries/PC6_B7.PDF.

Parikh, B.A., Tortora, A., Li, X.-P. and Tumer, M.E. 2008. Ricin Inhibits Activation of the Unfolded Protein Response by Preventing Splicing of the HAC1 mRNA. J. Biol. Chem. 283: 6145-6153.

Pettit, G.R. 1977. Biosynthetic products for cancer chemotherapy. vol. 1. Plenum Press. New York.

Phillips, R. and Martyn, R. 1999. Annuals and Biennials. p106, Macmillan, London

Poli, M.A., Rivera, V.R., Hewetson, J.F. and Merrill, G.A. 1994. Detection of ricin by cholorimetric and chemiluminescence ELISA. Toxicon 32: 1371-1377.

Purushotham, N.P., Raghavan, G.V., Rao, M.S., Reddy, M.R. and Mahender, M. 1985a. Studies on the pathology of experimental feeding of castor bean meal (*Ricinus communis*) in sheep. Indian Vet J 62: 116-118.

Purushotham, N.P., Veeraraghavan, G., Naidu, M.M. and Mahender, M. 1985b. Haematological studies on experimental feeding of castor bean meal (*Ricinus communis*) in sheep. Indian Vet J 62: 379-382.

Purushotham, N.P., Rao, M.S. and Raghavan, G.V. 1986. Utilization of castor bean meal in the concentrate mixture of sheep. Indian J Anim Sci 56 (10): 1090-1093.

Puttaraj, S., Bhagya S., Murthy, K. N. and Singh, N. 1994. Effect of detoxification of castor seed (Ricinus communis) protein isolate on its nutritional quality. Plant Foods for Human Nutrition 46: 63-70.

Ramakrishnan, S., Eagle, M.R. and Houston, L.L. 1982. Radioimmunoassay for ricin A- and B-chains applied to samples of ricin A-chain prepared by chromatofocussing and by DEAE Bio-gel A chromatography. Biochem. Biophys. Acta 719: 341-348.

Rao, M.S., Purushotham, N.P., Raghavan, G.V. and Mahender, M. 1984. Biochhemical changes in experimemntal feeding of castor bean meal (Ricinus communis) in sheep. Indian J Vet Pathol, 8: 33-36.

Rao, N.P., Reddy, B.S. and Reddy, M.R. 1988. Utilization of autoclaved castor bean meal in cancentrate ration for sheep. Indian Journal of Animal Nutrition 5: 121–128.

Rauber, A. and Heard, J. 1985. Castor bean toxicity re-examined: a new perspective. Vet.Hum.Toxicol. 27: 498-502.

Robb, J.G., Laben, R.C., Walker, Jr., H.G. and Herring, V. 1974. Castor meal in dairy rations. Journal of Dairy Science 57: 443-449.

Roberts, L.M. and Smith, D.C. 2004. Ricin: the endoplasmic reticulum connection. Toxicon 44: 469-472.

Roth, L., Daunderer, M. and Kormann, K. 1994. Giftpflanzen – pflanzengifte. Vorkommen, wirkung, therapie, allergische und phototoxische reaktionen. 4th edition. ECOMED, verlagsgesellschaft AG & Co., Landsberg, pp. 277-278.

Rubina, A.Yu., Dyukova, V.I., Dementieva, E.I., Stomakhin, A.A., Nesmeyanov, V.A., Grishin, E.V. and Zasedatelev, A.S. 2005. Quantitative immunoassay of biotoxins on hydrogel-based protein microchip. Anal. Biochem. 340: 317-329.

Rutenber, E. and Robertus, J.D. 1991. The structure of ricin B chain at 2.5A resolution. Proteins 10: 260-269.

Rutenber, E., Katzin, B. J., Collins, E.J., Mlsna, D., Ernst, S., Ready, M.P. and Rubertus, J.D. 1991. The crystallographic refinement of ricin at 2.8A resolution. Proteins 10: 240-250.

Sadani,G.R., Soman,C.S., Deodhar,K.K., and Nadkarni,G.D. 1997. Reactive oxygen species involvement in ricin-induced thyroid toxicity in rat. Hum.Exp.Toxicol. 16, 254-256.

Seto, Y. and Kanamori-Kataoka, M. 2005. Mass spectrometric strategy for the determination of natural and synthetic organic toxins. J. Health SDci. 51: 519-525.

Shankar, K., Zeng, K., Ruan, C. and Grimes, C. 2005. Quantification of ricin concentrations in aqueous media. Sensors and Actuators B 107: 640-648.

Soto-Blanco, B., Sinhorini, I.L., Gorniak, S.L. and Schumacher-Henriqure, B. 2002. Ricinus communis cake poisoning in a dog. Vet. Hum. Toxicol. 44: 155-156.

Sperti, S., Montanaro, L., Mattioli, A., Testoni, G. and Stirpe, F. 1976. Inhibition of protein synthesis in vitro by crotins and ricin; effect on the steps of peptide chain elongation Biochem. J. 156: 7-13.

Stein, S., Adamik, K and Kohn, B. 2006. Ricinintoxikation bei zwei Hunden. Kleintierpraxis, 51 (2): 89-92.

Stillmark, H. 1888. Ûber Ricin, eines gifiges Ferment aus den Samen von Ricinus communis L. und anderen Euphorbiacen. Inaugiral Dissertation, University of Dorpat; Eustonia.

Stirpe, F., Pession-Brizzi, A., Lorenzoni, E., Strochi, P., Montanaro, L. and Sperti, S. 1976. Studies on the protein from the seeds of Croton tiglium and Jatropha curcas. Toxic properties and inhibition of protein synthesis in vitro. Biochem J. 156: 1-6.

Stirpe, F. and Battelli, M.G. 2006. Ribosome-inactivating proteins: progress and problems. Cell Mol.Life Sci. 63: 1850-1866.

Tang, G., Minghuang, C., HongJing, W., Tang, G.Y., Chen, M.H. and Wu, H.J. 1994. Isolation and determination of croton toxin by high performance gel filtration chromatography (GFC) with photodiode array detector. Chinese Journal of Chromatography 12: 244-246.

Tokarnia, C.H., Doberenier, J. and Peixoto, P.V. 2002. Poisonous plants affecting livestock in Brazil. Toxicon 40: 1635-1660.

Tyler, V.E., Brady, L.R. and Robbers, J.E. 1976. Pharmacognosy, 7th ed. Philadelphia, Lea and Febiger, pp. 118.

Utskarpen, A., Slagsvold, H.H., Iversen, T.G., Wälchli, S. and Sandvig, K. 2006. Transport of ricin from endosomes to the Golgi Apparatus is regulated by Rab6A and Rab6A. Traffic 7: 663–672.

Van Raamsdonk, L.W.D. 2007. A survey on the presence of undesirable botanic substances in feed in the European Union. Report 2007.004. Rikilt Institute of Food Safety, Wageningen University and Research Centre, Wageningen, The Netherlands.

Vilhjalmsdottir, L. and Fisher, H. 1971. Castor bean meal as a protein source for chickens: Detoxification and determination of limiting amino acids. Journal of Nutrition 101: 1185-1191.

Völker, R. 1950. Fröhner´s Lehrbuch der Toxicologie für Tierärzte. 6th edn., Ferdinand Enke Verlag, Stuttgart.

Wales, R., Richardson, P.T., Roberts, L.M., Woodland, H.R. and Lord, J.M. 1991. Mutational analysis of the galactose binding ability of recombinant ricin B chain. J. Biol Chem. 266: 19172-19179.

Waller, G.R., Ebner, K.E., Scroggs, R.A., Das Gupta, B.R. and Corcoran, J.B. 1966. Studies on the toxic action of ricin. Proceedings of the Society for Experimental Biology and Medicine, 121: 685-691.

Wedin, G.P., Neal, J.S., Everson, G.W. and Krenzelok, E.P. 1986. Castor bean poisoning. The American journal of emergency medicine 4(3): 259–61.

Weiss, E.A. 2000. Castor. In "Oilseed Crops, 2nd Edition", Blackwell Scientific Ltd., Oxford, pp13-52.

Woo, B.H., Lee, J.T. and Lee, K.C. 1998. Purification of sepharose-unbinding ricin from castor beans (Ricinus communis) by hydroxyapatite Chromatoography. Prot. Expression and Purification 13: 150-154.

Woo, B.H., Lee, J.T., Na, D.H. and Lee, K.C. 2001. Sepharose-unbinding ricin E as a source for ricin A chain immunotoxin. J. Immunological Methods 249: 91-98.

Yang, Y.Y., Mon, D.H., Tong, Z.Y., Mu, X.H. and Hao, L.Q. 2007. Determination of ricin by double antibody sandwich enzyme-linked immunosorbent assay. Chinese Journal of Analytical Chemistry 35: 439-442.