RICIN FACT SHEET

1. Challenges in development of analytical methods for ricin have been considered by the Scientific Advisory Board since its Sixth Session, in particular through the Temporary Working Group on Sampling and Analysis (which held its final meeting in September 2012). In addition to being listed in Schedule 1 of the Chemical Weapons Convention, ricin is covered by the Biological and Toxins Weapons Convention.

2. A first version of a fact sheet on ricin was developed in 2012 and made available in the public domain (in Annex 5 of SAB-18/1, dated 19 April 2012).

3. For ease of reference by States Parties, the fact sheet (as published in 2012) is hereby issued under separate cover.

Annex:

Ricin Fact Sheet.

Annex

RICIN FACT SHEET

1.   Introduction

Ricin[1] is a potent proteinaceous toxin found in the seeds of the castor bean plant (Ricinus communis) (Figure 1). It is a controlled chemical under Schedule 1A of the Chemical Weapons Convention (CWC), and is a Category B substance under the Biological and Toxins Weapons Convention (BTWC). Ricin has attracted interest as a military chemical/biological warfare agent and as a poison for criminal and terrorist use.[2]

Figure 1: The castor bean plant Ricinus communis and its seeds (castor beans).
[Seed figure from Wikipedia]

2.   Structure

Ricin is a glycosylated protein consisting of two globular polypeptide chains, an A-chain and a B-chain containing 267 and 262 amino acid residues respectively, linked by a disulfide bond, and to which various glycoside (sugar) chains are attached.[3] Both chains are required for high toxicity (see below). Ricin has an approximate molecular mass of 65 kDa but is not a homogeneous chemical entity. Variations occur in the attached glycoside chains, and a small number of variants (isoforms) have been identified in the amino acid sequence of the polypeptide chains.[4] Genetically modified forms of ricin are possible. In response to a request from the

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[1]   CAS registry number as listed in Schedule 1, 9009-86-3

[2]   Ricin. Threat, effects and production. FOI User Report FOI-R-1261-SE (2004).

[3]   Lord J M, Roberts L M and Robertus J D. Ricin: structure, mode of action, and some current applications. *FASEB J*, 8, 201-208 (1994).

[4]   Despeyroux D, Walker N, Pearce M et al. Characterization of ricin heterogeneity by electrospray mass spectrometry, capillary electrophoresis, and resonant mirror. *Analytical Biochemistry*, 279, 23-36 (2000).

Director-General, the Scientific Advisory Board (SAB) proposed the following definition of ricin for verification purposes:[5]

(a) "All forms of ricin originating from Ricinus communis, including any variations in the structure of the molecule arising from natural processes, or man-made modification designed to maintain or enhance toxicity, are to be considered ricin as long as they conform to the basic 'native' bipartite molecular structure of ricin that is required for mammalian toxicity, i.e. A and B chains linked only by a disulfide bond (A-S-S-B). Once the inter-chain S-S bond is broken or the protein denatured it is no longer ricin."

3. Sources of ricin

3.1 The castor bean plant is widespread in hot climatic regions and is grown in temperate regions as an ornamental plant. It is cultivated industrially for the production of castor oil (used in a wide range of products including lubricants, hydraulic fluids, paints, textiles, polymers, and medically as a purgative). More than one million tonnes of seeds are processed annually. India, China and Brazil are the major producers of castor oil. The seeds typically contain 30-60% by weight of castor oil (predominantly ricinolate, a triglyceride of 12-hydroxyoleic acid); the ricin content is typically 1-5% by weight of the residual solid after removal of the oil. The oil is extracted by cold or more commonly hot hydraulic pressing, plus solvent extraction (hexane or heptane) of oil remaining in the mash. The residue from these processes is used as livestock feed or as fertiliser after the ricin has been deactivated by heating. Castor oil production plants are not subject to Schedule 1 inspections under Article VI of the CWC, but the SAB recommended that the Director-General encourage National Authorities in producing countries to promote hot pressing and other techniques that ensure inactivation of residual ricin in the waste mash.[6]

3.2 Several recipes for the isolation of ricin from castor seeds are available in the scientific literature and on the Internet. Crude ricin is easily prepared from ground seed by extractive removal of the oil with acetone, ether or hexane, and extraction of the water-soluble protein fraction into mildly acidified water (e.g. with acetic acid). If not detoxified, ricin and other proteins can be extracted into dilute acid from the waste mash from castor oil production, and precipitated with ammonium sulfate. The ricin can be purified by chromatography. The isolated ricin is a water-soluble white powder that is stable under normal ambient conditions.

4. Toxicity

The lethal doses of ricin in experimental animals by inhalation and by parental routes of administration are mostly in the low microgram per kilogram range, typically 1-10 µg/kg.[7] For comparison, the most toxic nerve agents would fall within the upper part of this range. A lethal dose of botulinum toxin A, the most potent bacterial toxin,

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[5] OPCW Scientific Advisory Board, SAB-14/1, 11 Nov 2009.

[6] OPCW Scientific Advisory Board, SAB-II/1, Section 2 and Annex 1

[7] Millard B and LeClaire R D. Ricin and related toxins: review and perspective. In: Romano J, Lukey B, Salem H (eds), *Chemical Warfare Agents 2nd Edition*, CRC Press, New York, 2008, pp. 423-467.

would be in the range 1-10 ng/kg. Inhalation toxicity is also likely to be in the range 1-10 μg/kg, depending on aerosol particle size, particles that are able to penetrate deep into the lungs (1-5 μm diameter) being considerably more toxic than larger particles.[8] Ricin is approximately 3 orders of magnitude less toxic by ingestion due to poor absorption from the gastro-intestinal tract, lethal doses being in the low mg/kg range. There are significant species differences in toxicity, up to 100 fold, rabbits being one of the more susceptible species. The human lethal dose by ingestion has been estimated as 1-20 mg/kg, and by injection 1-10 μg/kg, but no reliable data exists. There is no documented information on confirmed human exposure to ricin by inhalation, although a suspected poisoning by this route occurred in 2008.[9] Ricin is unlikely to be effective by contact with intact skin due to poor absorption. No toxicity was observed in mice dermally exposed to ricin at 50 μg per spot.[10]

5. Mechanism of action

The toxicity of ricin results from its ability to inhibit protein synthesis in eukaryotic cells.[11] It is part of a larger group of proteins known as type 2 ribosome-inactivating proteins (RIPs). This group includes other toxins from higher plants, e.g. abrin, pulchellin, modeccin, volkensin and viscumin, and some bacterial Shiga and Shiga-like toxins. RIPs have enzymatic N-glycosidase activity. They catalyse the hydrolytic cleavage of a specific N-glycoside bond between an adenine base and ribose residue of ribonucleic acid (RNA) in the 28S subunit of eukaryotic ribosomes. The catalytic glycosidase activity of ricin and similar toxins resides in the A-chain. The B-chain is a lectin (a sugar binding protein), which binds to terminal galactoside residues in sugar chains on cell surfaces. The attached A-chain is subsequently delivered to its ribosomal target inside the cell through a process of endocytosis, and after cleavage of the disulfide bond inside the cell. Both A and B chains are required for high toxicity. Following termination of protein synthesis a process of programmed cell death occurs (apoptosis).

6. Clinical features of ricin intoxication

6.1 Cell death following ricin intoxication may lead to tissue damage, organ failure and eventual death.[12] This progression generally occurs over 1-5 days in experimental animals and man, depending on the dose and route of intoxication. Clinical signs of poisoning and pathology reflect the organs most effected, which vary with the route of

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[8] Data submitted to the SAB Temporary Working Group seminar by Gareth Griffiths, CBD Porton Down, United Kingdom, in his presentation 'Toxicity of ricin (parenteral and inhalation routes)', 22–23 March 1999.

[9] Presumed accidental self-poisoning in a hotel room in Las Vegas, February 2008. Respiratory distress main symptom but route of exposure not confirmed.

[10] Franz D R and Jaax N K. Ricin toxin. In: Sidell F R, Takafuji E T, Franz D R (eds), *Medical Aspects of Chemical and Biological Warfare*, Office of the Surgeon General, Dept. of the Army, United States of America, 1997, pp. 631-642.

[11] Audi J, Bebon M, Patel M *et al*, Ricin poisoning a comprehensive review, *JAMA,* 294, 2342-2351 (2005).

[12] Bradberry S M, Lord J M, Rice P and Vale J A. Ricin and abrin poisoning. In: Marrs T C, Maynard R L, Sidell F R (eds), *Chemical Warfare Agents Toxicology and Treatment*, Second Edition, John Wiley & Sons Ltd, Chichester, UK, 2007, pp 613-632.

exposure. Many features can be explained by damage to endothelial cells resulting in fluid/protein leakage and tissue oedema (vascular leakage syndrome). Loss of appetite, lethargy and flu-like symptoms are common early signs.

6.2   Poisoning by ingestion is characterised by lesions of the gastro-intestinal tract, manifested by slow onset (hours) of vomiting, diarrhoea, gastric haemorrhaging, hypovolemic shock (from loss of blood volume) and organ failure, particularly of the spleen, liver and kidney. Depending on the dose, death may occur within 2-5 days. Injection of ricin produces swollen and haemorrhagic lymph nodes, severe internal bleeding and tissue damage, with the collapse of major organ systems. Inhalation of ricin causes slow onset (several hours) of respiratory distress (difficulty breathing), coughing, fever, pulmonary lesions and oedema. Depending on the severity of the exposure, respiratory failure and death may occur in 1.5-3 days. Exposure of the eyes causes severe irritation and conjunctivitis.

7.   Medical treatment

7.1   No approved antidote to ricin is currently available although vaccines are in development. Seven animal studies have shown that both passive administration of anti-ricin antibodies and active immunisation with a formalin-inactivated toxoid have the potential to protect against ricin challenge. At least one vaccine for active immunisation (for use in situations where a threat of exposure exists) has progressed to clinical trials, based on a recombinant A chain of ricin, modified to prevent vascular leakage through damage to blood vessel walls.

7.2   The progressive nature of ricin intoxication requires hospitalisation and continual supportive care. Medical treatment is focused on eliminating the toxin from the body as quickly as possible, and symptomatic and supportive treatment to minimise the effects of poisoning. Examples are flushing of the stomach with active charcoal in the case of ingestion, administration of intravenous fluids and electrolyte replacement in cases of severe dehydration and hypovolemic shock, and ventilatory support following inhalation. Following ingestion of castor beans, patients who receive prompt treatment are likely to survive (and even without treatment survival rates are generally high).

8.   Potential medical applications of ricin

Ricin was first shown to inhibit tumour growth in 1951. In more recent years it has been explored, as yet unsuccessfully, as the cytotoxic component of potential anti-tumour agents called immunotoxins. Ricin, or the catalytic A-chain (or genetic modifications thereof), is targeted at cancer cells by conjugation to a tumour cell-specific monoclonal antibody.[13] Ricin-based immunotoxins have also been studied for use in bone marrow transplants to destroy unwanted cells.

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[13]   Griffiths G, Leith A and Green M. Proteins that play Jeckyll and Hyde, *New Scientist*, 16 July 1987, 59-61.

9.   Prior military interest and weaponisation

Military interest in ricin arose from its relatively high toxicity (though significantly less than some bacterial toxins) combined with its widespread occurrence. For military use, other than for small scale poisoning, ricin would have to be disseminated as an inhalable aerosol, or possibly impregnated onto flechettes. It was investigated by the United States of America as a chemical agent towards the end of WW I but problems were encountered with thermal instability and aeroionisation using explosive dissemination. Further development and field trials during WW II, under the code name 'W', resulted in limited weaponisation in bombs.2 Iraq reportedly attempted to weaponise ricin in the 1980s. Ricin is not known to have been used as a military weapon so its effectiveness as a chemical/biological warfare agent is unproven. Some observers have questioned its utility as a military warfare agent.[14]

10.   Criminal and terrorist interest

10.1   The availability of castor beans, plus the relatively simple procedure for the isolation of crude ricin, has attracted criminal and terrorist interest for small scale poisoning, or for causing disruption (e.g. letters containing traces of ricin in a white powder). There have been many reported incidents of individuals possessing or attempting to isolate small amounts of ricin at home or in makeshift laboratories. In many cases the motive was murder of an individual.[15] Publicity given to instances of illegal possession or use, often with exaggerated claims of the hazard ricin presents, has added to public awareness.[16] Trace amounts of ricin, and documents describing its isolation, have been found in laboratories in Afghanistan.

10.2   The most widely publicised criminal use of ricin was the politically motivated assassination of the dissident Bulgarian journalist Gorgi Markov in London in 1978, following an attempted assassination two months earlier of a second Bulgarian exile in Paris by similar means. Ricin was administered by injection of a tiny engineered metal pellet from a compressed gas device hidden in an umbrella. Markov felt immediate pain at the site of injection in the thigh, with slowly developing fatigue, nausea, vomiting and fever. These signs progressed to necrotic swollen lymph nodes, gastro-intestinal haemorrhage, hypovolemic shock and renal failure after 36 h, with death on the third day. It was estimated that he had received a dose of ~500 µg although ricin was never isolated or confirmed analytically.[17]

11.   Detection and verification

Field detection

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[14]   See footnote 10.

[15]   See footnote 2.

[16]   Schep L J, Temple W A, Butt G A and Beasley M D. Ricin as weapon of mass terror – separating fact from fiction. *Environment International*, 35, 1267-1271 (2009).

[17]   Crompton R and Gall D. Georgi Markov – death in a pellet. *Medico-Legal Society Journal*, 48, 51-62 (1980).

11.1   Many developmental field detectors for biological agents and toxins have been reported to have the capability to detect ricin.[18] Most rely on molecular recognition by antibodies. Enzyme linked immunosorbent assay (ELISA) in various formats has been the most widely used, e.g. using colorimetric, electrochemiluminescent, or colloidal gold reporter systems. Other types of immunoassay include immunochromatographic hand-held devices, and more recently fluorescence based immunoassays using fibre optics or quantum dots. As an alternative to antibodies, molecular recognition based on aptamers (nucleic acid or peptide sequences with selective recognition properties) has been described for multiple agent detection including ricin, e.g. in a microarray format, or coupled with surface enhanced Raman spectroscopy.

Laboratory detection and identification

11.2   As a large biologically active molecule, laboratory screening for ricin as a powder or in aqueous solution can be performed using immunoassays and functional bioassays.[19],[20] Molecular recognition using different types of immunoassay, particularly ELISA, is widely used. ELISA is generally very sensitive (e.g. pg-ng/ml in aqueous solution) although cross reactivity with structurally related proteins can be a shortcoming. Functional bioassays have the advantage that they detect the biologically active molecule although specificity may be low. Examples of bioassays are toxicity in a small animal model or cell culture, and enzymatic activity (cleavage of an adenine residue) on an RNA preparation. The latter may be detected using fluorescence, electrochemiluminscence or mass spectrometric based assays. Specificity can be increased by using immunocapture to isolate the protein. Polymerase chain reaction (PCR) technology may identify the plant source of an environmental sample from residual traces of DNA from Ricinus communis, unless the ricin has been rigorously purified.

11.3   As a protein of known structure, ricin can be identified using mass spectrometry, usually in combination with liquid chromatography (LC-MS). Molecular masses are determined for the intact molecule, plus the mixture of smaller peptides ('peptide map') formed on selective digestion with enzymes such as pepsin or trypsin. The amino acid sequences of a selection of these peptides (minimum of three) are determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Identification is confirmed by searching against protein databases or by comparison with an authentic sample. The SAB Temporary Working Group (TWG) on Sampling and Analysis has recently addressed identification criteria for the verification of ricin.[21] For OPCW purposes, the TWG recommended that ricin should be identified by at least two different techniques, a screening technique, for example from the

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[18]   Ler S G, Lee F K and Gopalakrishnakone P. Trends in detection of warfare agents. Detection methods for ricin, *Staphylococcal enterotoxin* B and T-2 toxin. *Journal of Chromatography A*, 1133, 1-12 (2006).

[19]   Ezan E, Duriez E, Fenaille F and Becher F. Functional assays for ricin detection, in, *Detection of Biological Agents for the Prevention of Bioterrorism*, Banoub J (ed), NATO Science for Peace and Security Series A - Chemistry and Biology, 131-147, 2010.

[20]   Musshoff F and Madea B. Ricin poisoning and forensic toxicology. *Drug Testing & Analysis*, 1, 184-191 (2009).

[21]   Report of the Sixth Meeting of the SAB Temporary Working Group on Sampling and Analysis, The Hague, The Netherlands, 17-18 November 2011. SAB-17/1, Annex 2.

immunoassays and bioassays described above, and confirmatory identification using LC-MS and LC-MS/MS.

Biomedical samples

11.4   Ricin is rapidly excreted from the body, predominantly as smaller peptide metabolites; typically up to 90% is excreted within 24 h in experimental animals. ELISA has been used for the detection of intact ricin in blood and other tissues up to at least 48 h after exposure, and in swab samples from nasal mucosa following inhalation in experimental animals. Circulating antibodies may be detectable after approximately two weeks in subjects who survive ricin intoxication. Some forensic laboratories analyse for the alkaloid ricinine in gastric contents and urine as a biomarker of ricin intoxication.[22] Ricinine is a low molecular mass (164 Da) component of castor seeds (typically 0.05-0.3% by weight) and is usually present in crude ricin preparations. It is readily isolated from biological fluids and can be identified using LC-MS/MS at least up to four days following an intoxication.

12.   Decontamination

Ricin can be detoxified by heating at 80°C for 10 min or 50°C for 60 min.[23] Hot pressing denatured ricin completely in the seed mash as indicated by non-responsiveness to an antibody.[24] Cold pressing generally leaves residual ricin. Treatment with sodium hypochlorite (bleach) also rapidly deactivates the toxin. It was almost completely detoxified by Chlorox domestic bleach within 15 minutes.[25]

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[22]   Johnson R C, Lemire S W, Woolfitt A R et al., Quantification of ricinine in rat and human urine: a biomarker for ricin exposure. *Journal of Analytical Toxicology*, 29, 149-155 (2005)

[23]   Mackinnon P and Alderton M. An investigation of the degradation of the plant toxin, ricin. *Toxicon,* 38, 287-291 (2000).

[24]   Barnes D J, Baldwin B S and Braasch D A. Degradation of ricin in castor seed meal by temperature and chemical treatment. *Industrial Crops & Products*, 29, 509-515 (2009).

[25]   Cole K, Gaigalas A and Almeida J, Process monitoring the inactivation of ricin and model proteins by disinfectants using fluorescence and biological activity. *Biotechnology Progress,* 24, 784-791 (2008).