FOI-R--1261--SE
June 2004
ISSN 1104-9154
User report

Ricin

Threat, effects and protection

Susanne Lundberg, Lena Melin, Calle Nilsson and Pontus von Schoenberg

NBC Defence
SE-901 82 Umeå

SWEDISH DEFENCE RESEARCH AGENCY
NBC Defence
SE-902 82 Umeå

FOI-R--1261--SE
June 2004
ISSN 1650-1942
User report

Ricin

Threat, effects and protection

Susanne Lundberg, Lena Melin, Calle Nilsson and Pontus von Schoenberg

Issuing organization	Report number, ISRN	Report type
FOI – Swedish Defence Research Agency NBC Defence SE-901 82 Umeå	FOI-R--1261--SE	User report
	Programme Areas 3. NBC Defence and other hazardous substances	
	Month year June 2004	**Project no.** E4723
	General Research Areas	
	Subcategories 33. NBC Studies	

Author/s (editor/s)	Project manager
Susanne Lundberg Lena Melin Calle Nilsson Pontus von Schoenberg	Lena Melin
	Approved by Åke Sellström
	Sponsoring agency SEMA
	Scientifically and technically responsible The authors

Report title (In translation)

Ricin. Threat, effects and protection

Abstract (not more than 200 words)

Ricin is a protein toxin, which although it is derived from plants does not itself constitute a living material. It is therefore by definition a chemical weapon. Since ricin is a protein, its properties are in many aspects different from the classical chemical weapons (*i. e.* nerve agents, mustard gas) which are all small organic molecules. Ricin is a toxin of "average" toxicity – it is roughly 1000 times less toxic than botulinum toxin, and as toxic as some of the nerve agents.

Its potential as a chemical weapon was first illustrated in 1978, when ricin was used to assassinate the Bulgarian defector, Georgi Markov. The actual usage of ricin, resulting in casualties, is limited to this and one additional case. On several occasions, though, individuals have been convicted of possessing ricin. The last findings of ricin that have attracted attention occured in London in January 2003 when traces of ricin were found in an apartment and later on in the American postal system in 2003 and 2004.

Ricin has several of the properties which are demanded on a toxin for it to be considered a potential threat. The seeds, from which ricin is derived, are easily obtained since the plant has been naturalized in several tropical and warm regions throughout the world, and is also commonly cultivated because of its beautiful appearance. The active substance is readily obtained to a relatively low cost and with low demands on technical skills or apparatus. The toxicity is relatively high, but with minimum risk of causing harm to users during handling. It is stable for a long time at room temperature, which indicates that the protein is relatively heat-stable. Post-exposure antidotes are not available, and the vaccines that have so far passed the clinical tests have showed negative side effects. A few states have earlier included ricin in their offensive BCW programs, but on all known occasions it has been excluded due to a too low effectiveness compared to other agents. The risk that ricin is going to be used by states is therefore considered to be low. The properties of ricin might make it an attractive tool for terrorists and criminals though, and descriptions on how to manufacture and use ricin are circulating among such groups of individuals. The usage is in this context considered to be limited to a low-scale level. More research needs to be done concerning dispersion techniques as well as decontamination, which will facilitate assessment of ricin as an average-scale chemical weapon (*i. e* dispersion within closed areas). The psychological effects of average-scale usage is considered to be high because of the large attention ricin has been given lately.

Keywords

Ricin, history, dispersion, symptoms, detection, analysis, toxicity, terrorism, criminality

Further bibliographic information	Language English
ISSN 1650-1942	**Pages** p. 29
	Price acc. to pricelist

Utgivare	Rapportnummer, ISRN	Klassificering
Totalförsvarets Forskningsinstitut - FOI	FOI-R--1261--SE	Användarrapport
NBC-skydd	**Forskningsområde**	
901 82 Umeå	3. Skydd mot NBC och andra farliga ämnen	
	Månad, år	**Projektnummer**
	Juni 2004	E4784
	Verksamhetsgren	
	Delområde	
	33. NBC-studier	

Författare/redaktör	Projektledare
Susanne Lundberg	Lena Melin
Lena Melin	**Godkänd av**
Calle Nilsson	Åke Sellström
Pontus von Schoenberg	**Uppdragsgivare/kundbeteckning**
	Krisberedskapsmyndigheten
	Tekniskt och/eller vetenskapligt ansvarig
	Författarna

Rapportens titel

Ricin. Hot, verkan och skydd

Sammanfattning (högst 200 ord)

Ricin är ett proteintoxin som fastän det framställs från växter i sig inte utgör ett levande material, och den korrekta definitionen är därför ett kemiskt stridsmedel. Eftersom det är ett protein skiljer det sig i många avseenden dock väsentligt från klassiska kemiska vapen som senapsgas och nervgaser. Ricin är ”medelgiftigt”; det är ca 1000 gånger mindre giftigt än botulinustoxin (ett proteintoxin som renas från bakterier) och ungefär lika giftigt som nervgaser. Ricins potential som kemiskt vapen illustrerades första gången 1978, då Georgi Markov injicerades med en dos som förmodas vara ricin, och senare avled. Trots den stora uppmärksamhet som ricin fått i media på senare tid liksom ett antal fynd som gjorts, begränsas avsiktligt användande med dödlig utgång till detta och ett ytterligare fall. Vid flera tillfällen har polisen, främst i USA, gripit personer som tillverkat ricin i mindre mängder. De senaste beslagen som fått stor uppmärksamhet var i London 2003 där man fann rester från ricintillverkning, liksom fynd i det amerikanska posthanteringssystemet 2003 och 2004. Ricin har många av de egenskaper som krävs för att ett toxin ska utgöra ett potentiellt hot. Utgångsmaterialet, d v s fröerna är lättillgängliga eftersom ricinbusken i vilt tillstånd är mycket utbredd på sydligare breddgrader och dessutom nått stor popularitet som prydnadsväxt. Den aktiva substansen går att framställa till låg kostnad, med mycket enkla hjälpmedel och utan avancerad teknisk kompetens. Toxinet har hög toxicitet utan att utgöra ett direkt hot vid framställning och lagring. Det håller sig intakt under en längre tid vid rumstemperatur, vilket indikerar att proteinet är relativt värmestabilt. Motmedel efter förgiftning saknas och de två vacciner som visat sig verkningsfulla är inte helt ofarliga. Några stater har tidigare haft med ricin som en del av ett offensivt B/C-vapenprogram, men i alla kända fall har det strukits på grund av en alltför låg potential relativt andra stridsmedel. Risken att ricin skulle användas av statliga aktörer bedöms därför som låg. Ricins egenskaper kan däremot göra det attraktivt för terrorister och kriminella och i många sådana kretsar cirkulerar beskrivningar över både tillverkning och utspridning. Användandet bedöms i de här sammanhangen främst begränsa sig till småskalig användning. Mer forskning behöver ägnas åt aerosolutspridning och sanering, vilket kan underlätta bedömningen av ricin som ett potentiellt vapen för medelskalig användning (exempelvis spridning i slutna rum). Den psykologiska effekten av medelskalig användning bedöms vara hög beroende på den uppmärksamhet som ricin fått på senare tid.

Nyckelord

Ricin, historik, spridning, symtom, indikering, analys, toxicitet, terrorism, kriminalitet

Övriga bibliografiska uppgifter	Språk Engelska
ISSN 1650-1942	**Antal sidor:** s. 29
Distribution enligt missiv	**Pris: Enligt prislista**

Contents

Summary

Ricin, a protein toxin, is under the Chemical Weapons Convention (CWC) defined as a Schedule 1 List (chemical agents) controlled substance, and under the Biological Toxins Weapons Convention (BTCW) as a Category B (biological agents/toxins) substance. As a weapon, ricin cannot be compared to biological warfare agents since it is not a living material. Thus, it can neither propagate nor spread from person to person. However, it is still a chemical warfare agent and differs in many ways from classic chemical warfare agents such as mustard gas and nerve gases; ricin being the most toxic one. Since it has no skin penetrating abilities, a more advanced dispersion technique is required. Nevertheless, much to the advantage of the perpetrators, this is favourable since the risk of being poisoned when handling it decreases. There are other protein toxins - mainly originating from bacteria - whose toxicity is higher than that of ricin. However, the lower toxicity is partly compensated by the wide availability of ricin seeds, and the simple methodology required for producing clean enough ricin.

Ricin showed the potential as an assassination weapon in 1978, when Georgi Markov was thought to have been injected with a dose of ricin. He died on the third day after the injury was inflicted. Despite much media coverage of ricin lately, the intentional use has been limited to a few cases, of which only the above mentioned and an additional one have resulted in casualties. On numerous occasions, single individuals or groups have been arrested for having manufactured smaller amounts of ricin, thus pointing to an unwanted interest in the toxin. The confiscation in London, in January 2003, when traces of ricin were found, received extensive media coverage, as well as a ricin letter sent to the White House and the finding of a small container with ricin that was processed at a postal facility in Greenville, South Carolina at the end of 2003.

Ricin can be extracted from the water-soluble part of castor beans containing approximately 1 % ricin. Annually, one million metric tons of castor beans are processed to produce castor oil. Thus, the castor oil production can theoretically generate approximately 10,000 tons of ricin. Neither the production of castor oil, nor that of its by-products, is regulated or surveyed. The oil is produced using a hot-press method, minimizing the amount of active toxin.

Ricin is easily produced and toxic if inhaled, ingested or injected. The lethal inhalation dose for an adult is approximately 0.5 mg. Following ingestion, toxicity is considerably lower than after inhalation. Probable scenarios would be administering injections on an individual scale, and a large-scale dissemination of ricin using an aerosol. There is no known antidote; however, ricin vaccine is currently available. Recent scientific work done on ricin has made possible the introduction of an improved vaccine.

Ricin has been part of the offensive biological and chemical warfare programs in some countries, but has in all known cases been omitted due to its shortcomings in relation to other agents. The risk of ricin being used by state actors is therefore considered to be low. None the less, the properties of ricin could attract the attention of terrorists and criminals, and ricin-making instructions and dispersion methods are currently circulating within many of those. However, ricin would most likely be used on a small-scale weapon, or even an individual scale. The psychological effects of a medium-scale attack could be assumed to be extensive due to the attention given to ricin lately.

Introduction

The toxin can be extracted from the seeds of the ricin plant growing in many parts of the world. Besides being a houseplant, it is also used in the production of castor oil. The US military interest in ricin and its possible use as a weapon dates back to the end of World War I. During World War II, a so-called W-bomb was developed in cooperation with Great Britain. The weapon was tested in the field, but never used and the project was discontinued. Moreover, ricin has been used as a murder weapon by various intelligence agencies, the most famous case being that of the Bulgarian defector Georgi Markov, who was assassinated in London in 1978. The extracted toxin ricin is listed in the CWC's Schedule 1 List. In accordance with the list, it is allowed to posses seeds or reduced (inactive) ricin.

For a couple of years now, the most disturbing thing has been that of terrorists having the potential for using biological agents. Anthrax is a hot topic, much due to the powder letters causing the death of five people and more than 18 cases of illness[1] in the US in the autumn of 2001. In the aftermath of the September 11 attacks and the first suspicions that al-Qaeda was the perpetrators behind the anthrax letters, ricin also became a topic of interest. The American investigations into al-Qaeda bases and training camps in Afghanistan that autumn generated numerous reports claiming findings of various chemicals and biological agents. Among other things, ricin-making instructions and alleged traces of ricin were found. However, no accounts proving actual production in Afghanistan have been published. Ricin made the headlines again in January and October 2003. In January, police arrested seven North African men at a London address. In their apartment, production equipment was found as well as remnants from ricin production. In October, a sealed container with ricin was processed at a postal facility in Greenville, South Carolina.

Besides the fact that ricin has been of current interest in connection with these events, research is on-going as regards the use of ricin for medical purposes, particularly in the fight against cancer. Unwanted side-effects - not previously investigated – are now identified and supervised. Even though it is not yet established whether ricin could be of medical use, the discovery regarding ricin and similar toxins improved functioning as immunotoxins, is said to be the most important one since the beginning of the 1980s.

The aim of the present report is to give an overall picture of current knowledge on ricin, its applications, analysis methods and effects following dispersion.

A report in Swedish was printed in June 2003 and was reviewed by Research Director Gertrud Puu, the Swedish Defence Research Agency, the Division of NBC, at a report seminar that took place May 26, 2003. Because of new events that occurred between May 2003 and June 2004, some of the text has been revised and a new review was made in June 2004.

The following areas of responsibility are represented in the report.

Susanne Lundberg	Effects and protection
Lena Melin	State and non-state actors
Calle Nilsson	Verification and analysis
Pontus von Schoenberg	Dispersion calculations

[1] According to the criteria stated by the CDC (Centers for Disease Control and Prevention), fall 2001, www.cdc.gov.

1. General properties

Proliferation and availability

Ricin can be derived from the seeds of the castor bean plant, *Ricinus communis* (Figure 1), a fast growing plant. It is a native of tropical Africa and the Middle East, but is today more or less growing wild in all subtropical or tropical climate regions. It can also be cultivated in colder climates, but then as an annual. It is a popular houseplant and can be seen in many parks, for example in Sweden. Depending on climate, the plant - occurring as a small bush or a tree - can grow up to 20 feet high.

Figure 1. Ricin plant and ricin seed. 3 mg of ricin can be extracted from each seed.

Ricin is present in all parts of the plant; however, the beans are by far the most toxic. Due to its high toxicity, warnings have been issued regarding cultivation of the plant, for example in Australia, Canada and the US. It is thus recommended not to cultivate it close to play grounds and nurseries; due to the colourfulness and attractive appearance of the beans, children might consume them. In the US, the castor oil production has been discontinued for quite some time, partly due to the high toxicity of ricin, and also due to the allergens present in the waste mash generated in castor oil production. However, the genetic information necessary for synthesising ricin, and the allergen in the plant were recently identified.

Consequently, the possibility - with the help of biotechnology - of growing ricin plants where these genes have been removed has renewed interest among people for the oil production in the US[2].

Being popular as houseplants, some Swedish botanical gardens have them in stock, even though a few have decided on dropping castor beans due to their high toxicity. Following the arrests of suspicious terrorists in London in 2003, English plant sales outlets decided on dropping both ricin plants and beans from their stock in order to limit the availability.

Production and fields of application

The ricin plant grows in many places around the world but is also cultivated for castor oil. The world production has steadily increased since the beginning of the 1960s, when FAO (Food and Agriculture Organization of the United Nations) statistics was introduced. In 2002, the world production of oil amounted to approx. 525 000 tons (Figure 2). The production of seeds has nearly doubled in quantity, and the estimated total content of ricin in the seeds crops for 2002 indicates that it is possible to extract approx. 10 000 tons of ricin from them. However, the main part is destroyed in the actual production process (see below).

[2] USDA Agricultural Research Service, ARS News & Information, *High-tech castor plants may open door to domestic production,* http://www.ars.usda.gov/is/AR/archive/jan01/plant 0101.htm, 05/23/03

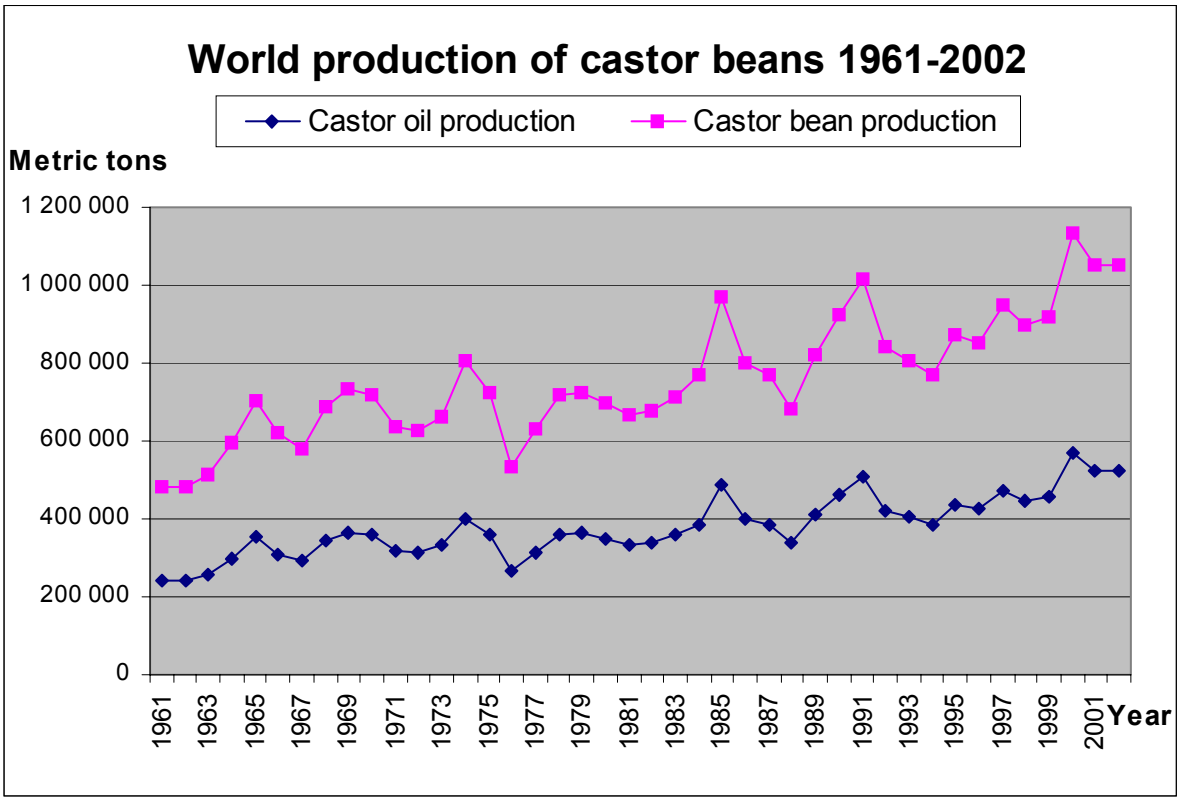

Figure 2. World production of castor beans and oil (Source: FAO[3])

For obvious reasons, the statistics from FAO covering some countries is not complete. Also, data is often missing, for example from Afghanistan, Iran, Iraq and North Korea, all having climatological prerequisites for a production. In this context, it is worth noting that the production quantities are similar for castor oil and linseed oil, whose world production amounted to 680 000 tons in 2002. India (60 %), China (24 %) and Brazil (9 %)[4] are the biggest producers of castor oil.

There is a very large variety of ricin plants – wild or crossed, with the aim of obtaining houseplants because of their beautiful foliage. Also, the large variety is considerable in commercial cultivation since different varieties require different climates and soils.

In the extraction of castor oil, manufacturers mostly use the hot-press method yielding a better profit than that of the cold-press method since the latter is often followed by an extraction stage including hexane, in order to extract remaining (5-19 %) oil from the waste mash. The cold-press method is also used, in which case the following heat treatment of the waste mash is of importance. The risk of waste mash or seeds going astray, and as such constituting the source material of potential illegal ricin extraction, the oil production can hardly be ignored considering the substantial production volumes. It is worth noting that civilian usage is perfectly legal, see chapter 2, *Verification and analysis*.

From World War I up until the 1960s when production of synthetic oils started, the oil was used in aircraft engines. The castor oil can also be improved further in order to be of use in industry and, too, in the production of for example nylon, lubricants, drugs, textiles, cosmetics and perfumes.

[3] Food and Agriculture Organization of the United Nations. FAOSTAT Agricultural data, http://apps.fao.org/page/collections?subset=agriculture.
[4] Production in 2002

For some time now, ricin has been of current interest in medical applications. In the beginning of the 1950s, scientists started to study whether ricin could possibly form the basis of cancer treatments. Over the last few years, the possibility of using ricin in HIV treatment has been investigated. In clinical tests, ricin, fused together with a "cell recognising molecule" (so-called immunotoxins), has proven to be of use in the treatment of certain types of cancer, for example leukaemia. A deglycosylated form of the protein chain containing the enzymatic activity is used (see below under "Chemical properties and results mechanisms"). Approximately 30 % of the patients responded well to the experimental treatment, but unfortunately some developed a symptom called VLS (Vascular Leak Syndrome). Through minor modifications in the protein, a variety of ricin not having this side-effect has successfully been produced. Therefore, it can be administered in higher doses, resulting in a favourable effect. Nevertheless, it has been questioned whether the recovery rates are sufficient for giving ricin cancer treatment status. Reportedly, this is the most important discovery since the beginning of the 1980s, with respect to ricin and similar toxins functioning as immunotoxins. Additional clinical trials will prove whether this is correct or not[5] [6].

Chemical properties and results mechanisms

As previously mentioned, there are different subspecies of the ricin bush, all producing seeds in two sizes. The smaller one - and the most common - weighs approx. 0.3 grams. Ricin is not a homogenous protein, but consists of at least two isoforms[7] [8] (variation on the amino acid level) and an unknown amount of glycoforms[9] (variation on the glycosylation level). Which of the isoforms the seed contains mainly depends on which of the subspecies it represents[10], but even the level of maturation of the seed and the cultivation conditions might be of importance in this context.

Independent of isoform, ricin is a 65 kD protein consisting of two subunits of approximately the same size, named A and B. The chains are joined by a covalent bond, a disulfide bridge, i.e. the chains dissociate from one another under reduced conditions. In this form, the protein is no longer toxic to humans. The fact that the protein is glycosylated (i.e. carbohydrate chains are linked to the protein) is thought to contribute to the protein stability and, too, that it might be crucial for the transport of the toxin within the cell.
The B chain binds to a sugar structure (galactose) present in high amounts on all cell surfaces. The chains are both taken up by the cell, and through a very complicated network of transport mechanisms in the cell, a small fraction of the ricin originally taken up, is transported to the protein synthesizing machinery of the cell (=ribosome) (Figure 3). In this process, the sugar binding function of the protein participates, but also other assisting proteins within the cell. When the ricin reaches the ribosome, the synthesis of new proteins is discontinued with the

[5] J.E. Smallshaw, B. Ghetie, J. Rizo, J.R. Fulmer, L.L. Trahan. M-A. Ghetie and E.S. Vitetta, *Nature Biotech.* 2003;21:387-391 Genetic engineering of an immunotoxin to eliminate pulmonary vascular leak in mice
[6] R.J. Kreitman *Nature Biotech*. 2003;21:372-374 (N&W) Taming ricin toxin
[7] J.W. Tregear and L-M. Roberts *Plant Mol. Biol.* 1992;18:515-525 The lectin gene family of *Ricinus communis:* Cloning of a functional ricin gene and three lectin pseudogenes
[8] T. Araki and G. Funatsu *Biochem. Biophys. Acta* 1987;911:191-200 The complete amino acid sequence of the B chain of ricin E isolated from small-grain castor bean seeds. Ricin E is a gene recombination product of ricin D and *Ricinus communis* agglutinin
[9] Y. Kimura, S. Hase, Y. Kobayashi, Y. Kyogoku, T. Ikenaka and G. Funatsu *J. Biochem.* 1988;103:944-949 Structures of sugar chains of ricin D
[10] D. Despeyroux, N. Walker, M. Pearce, M. Fisher, M. Mcdonnell, S.C. Bailey, G.D. Griffiths and P. Watts *Anal. Biochem.* 2000;279:23-36 Characterization of ricin heterogeneity by electrospray mass spectrometry, capillary electrophoresis and resonant mirror

help of a chemical reaction triggered by the protein. Since the reproduction of proteins no longer can occur, the cell will die in a time-span that varies depending on cell type[11] [12].

This mechanism is not unique for ricin. It belongs to a family of toxins usually named RIP (Ribosome Inactivating Proteins), and one distinguishes between RIP I and RIP II. The RIP I proteins consist solely of an A chain and is as such only taken up by cells to a very limited extent. The RIP II proteins consist of chain A and chain B. Accordingly, only the RIP II is toxic to humans. The most characterized RIP II protein beside ricin is abrin (Rosary pea plant *Abrus precatorius*), viscumine (mistletoe *Viscum album*), modeccine (*Adenia digitata)* and volkensine (*Adenia volkensii)*[13]. Abrin is extracted and works in almost the same way as ricin, however, it is three times more toxic to mice[14], according to toxicological data. The mechanism of using part of the protein in order to bind to cells and the other to execute the enzymatic activity necessary is not unique for this group of proteins; the technique is also applied by a large amount of other toxins. For example, certain toxins from *E. coli* and *Shigella* can also inactivate the protein synthesis in a cell after having been taken up by the cell in a similar way to ricin[15].

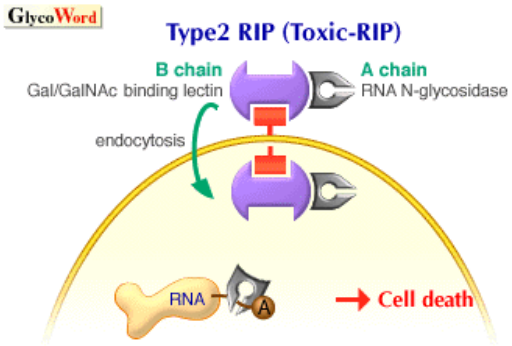

Figure 3. Schematic picture of ricin poisoning of a cell. The B chain bind to the cell surface and with this, both the A and B chains are taken up by the cell (GlycoWord; Lectins (Hanae Kaku))

Production

Ricin is a protein which is soluable in water and is extracted, for example, by homogenising seeds using an acid water solution.
The water extract is thus crude, that is, many additional proteins and smaller components are present. In the case when a so-called "crude extract" was analyzed with respect to toxicity, it was clear that the toxicity was 50 % lower compared to that of clean material. The report does not state how this "crude extract" has been produced, but most probably it is a water extract.

In the production of pure ricin, a number of conventional chromatographic stages must be carried out, skills available to a chemistry graduate. In our hands, the yield with respect to pure ricin, is 4-5 mg/g of ricin seeds, but most probably it is at least possible to reach a twice as high yield, if no considerations to the degree of purity is taken (i.e. using an extract having not been further processed). In a recent publication, describing a simplified method for ricin purification (amongst other things originating from an acetone extract instead of a water extract), the yield was 0.9 % from the plant extract (corresponding to 10 mg/g of seeds)[16].

[11] J.M. Lord, L.M. Roberts and J.D. Robertus *FASEB J.* 1994;8:201-208 Ricin: structure, mode of action, and some current applications
[12] S. Olsnes and J.V. Kozlov *Toxicon* 2001;39:1723-1728 Ricin
[13] F. Stirpe, L. Barbieri, M.G. Battelli, M. Soria and d.A. Lappi *Biotechnology* 1992;10:405-412 Ribosome-inactivating proteins from plants; present status and future prospects
[14] S. Olsnes and A. Pihl *Eur. J. Biochem.* 1973;35:179-185 isolation and properties of abrin: a toxic protein inhibiting protein synthesis. Evidence for different biological functions of its two constituent peptide chains
[15] K. Sandvig and B. van Deurs *EMBO J.* 2000;19:5943-5950 Entry of ricin and shiga toxin into cells: molecular mechanisms and medical perspectives
[16] S.M. Darby, M.L. Miller and R.O. Allen *J. Forensic Sci.* 2001;46(5):1033-1042 Forensic determination of ricin and the alkaloid marker ricinine from castor bean extracts

2. Verification and analysis

Chemical Weapons Convention and ricin

Ricin and saxitoxin were the two first chemical substances from living material to be listed by the Chemical Weapons Convention (CWC, Schedule 1) because, by definition, they are chemical warfare agents despite their complex nature and origin. Since part of the work is performed in order to secure the conformity to the CWC, analytical methods for unambiguous identification must be available at the designated laboratories classified as qualified in this context. Thus, the demands on these laboratories are heightened.

The Chemical Weapons Convention prescribes extracted, active ricin[17], possession of seeds is thus not a criminal offence. At the same time, reduced ricin (when the two in-going protein chains have been separated from each other) can be handled and used for research purposes without any restrictions. Precursors of chemical warfare agents are listed on Schedule 2 of the CWC. For ricin it could be argued that the seeds should have been included in that list which however would have given rise to unreasonable consequences regarding the production of castor oil. Instead it is recommended that countries, in which castor oil is produced, must be urged to use the hot-press method or to inactivate ricin in another way.[18]

When it comes to medical research on ricin (immunotoxins) and vaccine development, one would most likely not make use of native ricin to such an extent that the CWC provisions would be violated. The ricin used in these cases will most likely be in an inactivated form. This contradiction has been validated in 2003 (see "Production and fields of applications" and "Medical protection").

Work to implement the obligations of the CWC

The criteria for verification set up by the OPCW implies some problems when comes to protein verification. The OPCW requirements[19] generally states that "The identification of each of the test chemicals must be based on at least two different analytical techniques giving consistent results; at least one of these techniques must be a spectrometric technique (e.g. EI/MS, CI/MS, LC/MS, NMR)." These requirements are generally suited for other listed compounds than proteins, and the fact that ricin is not a single compound, due to the isoforms from variation in the amino acid backbone of the protein and also the variation introduced by variations in glycosylation, implies that a different approach must be taken.

Analysis methods

Screening

Antibodies against ricin are commercially available. However, these are often produced using a closely related protein, ricin agglutinin (RCA), which results in the production of less specific antibodies giving cross reactions with other proteins. This considerably increases the possibility of false positive answers in an assay like ELISA. However, such assays are of great value for screening purposes and might be improved by the usage of more specific antibodies. An advantage of unspecific antibodies is however, that they can "catch" the presence of other similar toxic proteins.

[17] Convention on the Prohibition of the Development, Production, Stockpiling and use of Chemical Weapons and on their Destruction. OPCW (1994).
[18] OPCW.Scietific Advisory Board, SAB-II/1, Annex 1, page 16, paragraph 6.4.
[19] Work instructions for the evaluation of the results of OPCW proficiency tests. Quality system document No.: QDOC/LAB/WI/PT3. Organisation for the Prohibition of Chemical Weapons Secretariat, 2003.

Liquid chromatography – mass spectrometry
In order to identify the presence of ricin in a suspicious sample molecular mass determination on an intact protein can be performed using mass spectrometry (MALDI-MS).

To verify the presence of a protein in a sample a general approach is to perform enzymatic degradation of the protein and analyse the peptide mixture by HPLC-MS in order to obtain molecular weight information for the peptides, a peptide map, which can be searched against available protein data bases. This will give a first identification of the protein. However, in order to achieve reliability in the verification of ricin, comparable to the requirements for other listed compounds, additional information must be obtained.

For proteins the conventional way to increase the level of information, which also is appropriate in this case,[20] is to run HPLC-MS/MS of the peptide mixture in order to obtain daughter ion spectra of the peptide molecular ions. These spectra give information about the amino acid sequence which then can be compared to database information also using the information about the enzyme used for digestion of the protein. Different enzymes have different targets for their action and cleave a protein at different positions. By this technique it is possible to gain a high coverage of the amino acid sequence of ricin and a possibility to identify peptide sequences that are unique to ricin. This is essential since other proteins, especially RCA which has a partially with ricin overlapping amino acid sequence, might be present in a sample. Since ricin comprises two protein chains (A and B), both needed for the in vivo toxic action, it must be demonstrated that both these are present. This means that e.g. two specific peptides for each of ricin A-chain and ricin B-chain should be identified in the sample in order to unequivocally verify the presence of ricin.

Functionality test
If required, functionality tests, i.e. proof that the toxin is active, can be added to the protocol. This can be accomplished by using a in vitro cell free activity test like the protein synthesis inhibiting test utilizing nuclease-treated rabbit reticulocyte lysate containing luciferase mRNA.[21] This test, however, requires in order being conclusive, the additional proof that ricin A-chain and ricin B-chain are still connected, since both are required for *in vivo* toxicity. This can be done by obtaining molecular mass information using MALDI-MS. An alternative would be to use a cell toxicity test in order to verify the toxins ability to enter the cell, as well as its protein inhibiting activity.[22]

Transportation, handling and analysis of ricin and other toxic samples, bring about risks and should be performed only by designated and specially equipped laboratories having the necessary experience.

[20] Fredriksson, S-Å., Hulst, A.G., Artursson, E., de Jong, A.L., Nilsson, C., van Baar, B.L.M. Forensic Identification of Neat Ricin and of Ricin from Crude Castor Bean Extracts by Mass Spectrometry. *Manuscript in preparation.*
[21] Hale, M. 'Microtiter-Based Assay for evaluating the Biological Activity of Ribosome-Inactivating Proteins.' Pharm. Toxicol. 88 (2991) 255-260.
[22] Battelli, M. G.; Musiani, S.; Monti, B.; Buonamici, L.; Sparapani, M.; Contestanile, A. And Stirpe, F. 'Ricin toxicity to mitoglial and monocytic cells.' Neurochem. Int. 39 (2001) 83-93.

Analysis of ricin – on-going research

For the past few years, FOI (Swedish Defence Research Agency), the Division of NBC Defence, has conducted research on reliable methods for analysis of ricin. The two iso-forms of the toxin have been purified from different subspecies of ricin, and properties such as binding to cells, amino acid sequencing, stability and toxicity have been investigated (in manuscript). The acquired knowledge within the field has not only been practiced in the requests for analysis obtained by the division with respect to ricin, but also in the international knowledge exchange with respect to protein toxins and, too, in the expert support offered to authorities and the general public when appropriate.

3. State and non-state actors

Offensive research programs

At the end of World War I, the US military took interest in the possibility of using ricin as a weapon, firstly due to the fact that there was an existent offensive biological warfare program and, secondly, due to the high toxicity and the fact that ricin can be relatively easily produced. During World War II - and under the code name "Compound W" - a W bomb was developed in cooperation with Great Britain. Even though the weapon was tested and found to be functional, it was never used in battle.

During the Cold War, ricin was used as an assassination weapon against defectors by Soviet Intelligence, because of its high toxicity and also to the limited possibility of effectively treating the poisoning. A few cases of that kind are described below under "Ricin as a weapon".

South Africa

During the South African apartheid regime, the BWC project "*Project Coast*" was performed. It was initiated in 1981 at the Ministry of Defence and was for the following twelve years supervised by the physician Wouter Basson, also called "Dr Death". The primary BC research was carried out at the Roodeplaat Research Laboratories, opened in 1983. There were also a number of research and testing centres at South African universities and companies, with scientists assisting the project.

Reportedly, "*Project Coast*" acquired and worked with anthrax, plague, cholera, *E. coli*, staphylococcus, ricin, botulinum, gangrene as well as Ebola, Marburg and Rift Valley viruses. A number of sources stated that entirely new pathogens were developed within the project. One of the main aims was to acquire - and test - different biological substances in order to develop protective means in the event of an anticipated attack with biological agents from the Soviet Union. Research on chemical and biological substances, intended to be used against the black community or opponents of the apartheid regime, was also carried out within the project. Also, research on race specific biological substances and production of drugs, such as ecstasy, was performed, in order to cause drug addiction within certain populations[23] [24].

Iraq

In 1995, after having only acknowledged the carrying out of "small scale defensive research" for four years, Iraq admitted to the production of, and also to have weaponized biological warfare agents, according to UNSCOM inspectors. However, only after having been confronted with having purchased large volumes of cultivation media, Iraq admitted to the allegations. Such information was also shared by the defector Husayn Kamil, formerly responsible for the Iraqi defence industry. Before the Gulf War, anthrax, botulinum toxin, afla toxin and ricin was produced and tested in the field using artillery grenades. Ricin research was on-going at the Salman Pak and Muthanna State Establishment facilities[25]. According to

[23] *Paths to disarmament: The Rollback of South Africa's Chemical-Biological Warfare and Nuclear Weapons Programs, Paper Presented at the 2001 Annual Meeting of the ISA,* Helen Purkitt, professor, Department of Political Science, U.S. Naval Academy and Stephen Burgess, Assistant Professor, Department of International Security Studies, U.S. Air War College

[24] *Project Coast: Apartheid's chemical and Biological Warfare Programme,* Chapter *Structure and management of Project Coast,* C. Gould & P. Folb, December 2002

[25] At Salman Pak, research was conducted on Anthrax, botulinum toxin, Clostridium perfringens, mycotoxins, aflatoxin and ricin in a laboratory scale. The Muthanna State Establishment was the facility initially used for the

the UNSCOM report to the UN Security Council in January 1999, Iraq acknowledged the production of 10 litres of ricin from 100 kg of seeds and that the whole quantity was used at one field trial in November 1990[26], with the use of 155 mm artillery grenades. The document received by UNSCOM at an inspection in 1997, indicated that far more than 100 kg of seeds had been processed in October-November 1990[27].

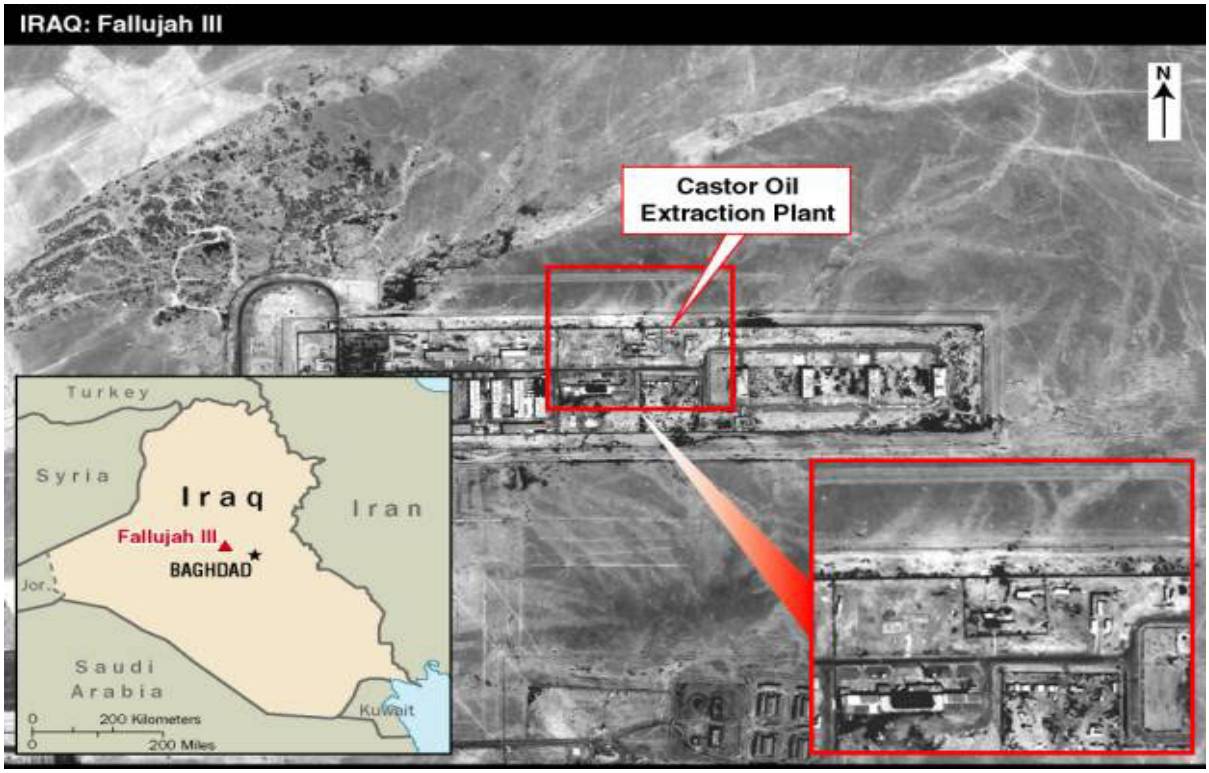

Figure 4. Castor oil plant Fallujah III outside Bagdad, Iraq (Photo:CIA)

In Fallujah III there was a facility historically connected to the Iraqi chemical warfare program. This "multi-purpose" facility was supposed to procure material to Muthanna. At the facility, Iraq legally produced castor oil under the close inspection of UNSCOM up until 1998 when the inspectors left Iraq. In 1999, Iraq began to reconstruct large parts of what was destroyed in Operation Desert Fox. At that point, the Iraqi authorities claimed that they produced castor oil with the intention of using it as break fluid[28].

Iraqi B weapons programme in 1985/86 but was primarily the main Iraqi facility for research and production of chemical weapons. The facility was in operation between 1983 and 1991 and produced thousands of tons of precursors, nerve gas and mustard gas. Muthanna was badly damaged during the Golf War and in 1992 to 1994 UNSCOM was working with the destruction of remaining material. Federation of American Scientists, *Major Sites Associated With Iraq's Past WMD Programs,* http://www.fas.org/news/un/iraq/s/971203_sites.htm

[26] There is a discrepancy between the amount of ricin possible to extract from this amount of seeds, and the actual amount that was produced: supposing that a ricin solution contains 10 mg/ml (our observation) at a maximum, 10 liter is equivalent to approx. 100 g ricin. According to calucaltions, 100 kg of ricin seeds will give approx. 1 kg of ricin

[27] Documents received at an inspection in 1997 indicate that far more than 100 kg ricin seeds were used during the period October – November 1990. Federation of American Scientists, *UNSCOM – Report to the Security Council – 25 January 1999,* article 83, http://www.fas.org/news/un/iraq/s/990125/dis-bio-htm

[28] Central Intellience Agency, *Iraq's Weapons of Mass Destruction Programs,* October 2002, http://www.globalsecurity.org/wmd/library/news/iraq/2002/Iraq_Oct_2002.htm

Ricin as a weapon

Even though deliberate use of ricin is not common, there are approximately twenty documented cases, in which perpetrators supposedly have acquired, planned to use or actually used ricin. However, no plans or attempts of large-scale production and dispersion have been revealed, it has all been about murder or murder attempts on one or numerous selected people. Thus far, a study of the known cases of usage shows that only two persons have died in ricin attacks since 1978. The compilation below of known cases is based on information in open sources, but it is unknown if extracted ricin were used or whether the perpetrators have been planning to use the unrefined castor beans.

State actors

Figure 5. Georgi Markov (Unknown photographer)

The most well-known cases would be the murders and attempted murders on four people in the 1970s, the victims being three Bulgarian defectors and one CIA double agent. In September 1978, Georgi Markov was attacked, who had since his defection in 1969 been living in London where he worked as a commentator for the Radio Free Europe. The assassin, most likely sent by the Soviet KGB, attacked Markov at a bus stop using a modified (ricin-rigged) umbrella as weapon, which injected a metal pellet containing ricin. Markov took hastily ill and died on the third day after the injury was inflicted.

The autopsy revealed a pinhead-sized hollow pellet (1.52 mm) with two perforated 0.34 mm holes embedded in his leg. The ricin dose in the pellet was estimated to be approximately 500 microgram. Markov's statement that the attacker picked up an umbrella from the ground after the attack, together with the fact that a drawing of an umbrella weapon was published, made the murder go under the name of "The Umbrella Assassination in London"[29] [30] [31].

Figure 6. The pellet found in Markov's leg (Unknown photographer)

The Bulgarian Vladimir Kostov had defected to France in June 1978 and worked as a correspondent for Bulgarian radio and television. In August 1978, two months prior to the attack on Markov, he was in the Paris Metro. He heard the sound like an airgun and immediately afterwards he felt a sting in the back. He took ill and was admitted to hospital where he stayed for twelve days, undergoing fever treatment. At the end of September, an X-ray revealed a pellet similar to that injected into Markov's leg. Tests of both pellets did not show presence of ricin or any other toxin. However, the victims' symptoms, along with performed animal tests, pointed to the fact that ricin was the toxin most likely to have been used[32].

In 1980, the CIA double agent Boris Korczak in McLean, Virginia, was attacked with a weapon similar to those used in the European attacks. Korczak was admitted to the hospital in

[29] Medico-Legal Society, Dr Rufus Crompton, *Georgi Markov – death in a pellet,* 03/13/80
[30] U.S. Army, Handbook of Military Medicine, *Chapter 32. Ricin toxin.* David R. Franz and Nancy K. Jaax
[31] Mitretek, http://www.mitretek.org/home.nsf/HomelandSecurity/Toxins
[32] Medico-Legal Society, Dr Rufus Crompton, *Georgi Markov – death in a pellet,* 03/13/80

Fairfax where he later died. As a result, the US/CIA and the Soviet Union started discussing who "owned" the body, since he was a Soviet citizen. When the cause of death was established, the Soviet Union claimed the deceased and brought the body to the USSR, the pellet stayed in the US[33].

Non-state actors

According to information in open sources, there are approximately twenty documented cases in which people or organisations allegedly have been in possession of home manufactured ricin. Also, there are other cases documented in which the perpetrator has not yet been able to actually manufacture ricin, but has had access to castor beans, production equipment and instructions. It is difficult to assess the amounts produced by various actors, mainly because of the authorities not wanting to go public with such information during on-going investigations. In the cases when ricin and beans were found, only the total amount was stated.

Single individuals and right-wing extremists

Single individuals, mostly acquainted with the victim, and having no connection to any organisations, have on numerous occasions tried to come across ricin and castor beans to be used in homicides. In right-wing circles ricin is considered to be a potent weapon, but no one has yet attempted to perform a ricin attack. A brief description of known incidents is given in Appendix 1.

al-Qaeda and Afghanistan

After the fall of the Taliban regime, and investigations made in Afghanistan as regards suspicious al-Qaeda camps and bases, many reports have been published on findings of various chemical and biological substances. Reportedly, in November 2001, reporters from The Times found, among other things, production manuals regarding ricin in an abandoned house in Kabul[34]. In March 2002, an article declared that traces of ricin had been found close to Kandahar[35]. In May 2004 it was reported that Menad Benchellali, known among his Arab friends as "the chemist", had spent some time in al-Qaeda training camps in Afghanistan. When he returned to France in 2001 he set up a laboratory in his parents' spare bedroom and began to manufacture ricin. The ricin was stored in small glass flasks and old jars of Nivea skin cream and according to some reports, similar bottles turned up in the UK in 2003. See "London 2003" below.[36]

Ansar al-Islam and Iraq

In August 2002, abcNews reported that the US had cancelled a planned raid on a Kurdish-controlled area in northern Iraq, in which area al-Qaeda sympathizers supposedly had been experimenting with poisonous gases and toxins. According to the article, the al-Qaeda adherents would be under the protection of the small, Islamic militant group Ansar al-Islam. Intelligence sources had supposedly told abcNews that; "Evidence will show that terrorists have tested ricin in water, as a powder and an aerosol". They used it to "kill monkeys, chickens and on one occasion an unwitting man was exposed at an Iraqi market place".

---

[33] Mitretek, http://www.mitretek.org/home.nsf/HomelandSecurity/Toxins
[34] Anthony Loyd and Martin Fletcher, *bin Laden's poison manual,* The Times, 11/16/01
[35] Courier Mail, *Troops find tiny traces of anthrax,* March 27, 2002
[36] NTI, *Work of Al-Qaeda "chemist" still interests European authorities*, 5/5/04, http://www.nti.org/d_newswire/issues/2004_5_5.html#74B3883F

Thereafter, they followed the man to watch him die several days later[37]. Whether the reports are true and verifiable is uncertain; the authorities have not presented any hard evidence backing up the story.

London 2003
In the beginning of January, seven North African men were arrested, under the suspicion of producing ricin in an apartment in a London suburb, in which production equipment and "small amounts of ricin remnants" were found. Since no ricin was found they were suspected of having hid the toxin elsewhere. One of the suspects had visited an al-Qaida camp in Afghanistan and reportedly, (in April 2003), the ricin could be linked to Ansar al-Islam in Iraq. According to reports in May 2004, the ricin could maybe be linked to Menad Benchellali, see "al-Qaeda and Afghanistan" above. Another suspect in London had a job with a company supplying food to a British military base. Hence, a likely scenario would be that of administering ricin into food[38] [39] [40]. Shortly following the arrests, English nurseries chose to drop ricin plants from their stock in order to make the availability more difficult.

Spain 2003
At the end of January, sixteen Algerians were arrested in Girona and Barcelona, suspected of being linked to al-Qaeda. At the time, the police found what was reported to be explosives, suspicious toxic material and manuals for chemical warfare. In British media, the chemicals were rapidly "identified" as ricin. In the reports regarding the event, the chemical findings were soon to be named "unidentified reports on ricin". At closer inspection, the press release in Spanish from the State Department did not refer to "ricin" but to "resina", the Spanish word for resin or rosin. Previously, three containers of powder and liquid had been sent for analysis, and the first analyses turned out to be "negative except for ricin". The final analysis answer only showed contents of aliphatic hydrocarbon in one sample and "substances present in resin and synthetic rubber" in the other one. Also, Spanish police had been granted to call in American expertise in order to analyse a powder for ricin in one of the apartments. The suspicious substance proved to be washing powder. The sixteen Algerians were all released at the end of April[41] [42].

France 2003
In March, five vials and bottles suspected of containing ricin were found stashed in a locker at the Gare de Lyon station in Paris. Reports published shortly after the findings, declared that the ricin was to have been mixed with "some other product"[43] in order to produce a highly toxic poison. However, the "amount of ricin was too small to be lethal". Another source stated that chemical analyses showed that "the ricin" matched the traces of that found in London in January 2003. In April 2003 - following analyses - an article was published claiming that the material found in the vials and bottles was in fact wheat and barley[44] [45] [46].

---

[37] abcNEWS, *Bush Cancels Iraqi Strike. Calls Off Operation to Take Out Al Qaeda-Sponsored Poison Gas Lab,* John McWethy, 08/20/02
[38] Reuters/New York Times, *Four are charged by Britain in toxin case,* 01/12/03, http://www.nytimes.com/2003/01/12/international/europe/12BRIT.html
[39] Maryann Bird, *A poisonous plot,* Time, 01/20/03
[40] Peter Finn, *Spain snares 16 alleged Al Qaeda operatives,* Washington Post, 01/24/03
[41] *Spanish judge awaiting ricin test before decision on al-Qa'idah suspects,* BBC Monitoring Europe, 02/14/03
[42] Justin Webster, *The strange case of the dangerous detergent: Whatever happened to the 16 alleged terrorists that Spain seized in January? Justin Webster reports from Catalonia,* New Statesman, 04/14/03
[43] Possibly acetone or ethanol according to *Terrorist attack seen in ricin in Paris,* The New York Times, 03/22/03
[44] *Paris: Ricin find 'non lethal',* CNN.com/World, 03/24/03

USA 2003 and 2004

In October 2003, an envelope containing a threatening note and a sealed container was processed at a postal facility in Greenville, South Carolina. The letter, which was addressed to the Transportation Department and signed by the signature "Fallen Angel", said that large amounts of ricin would be used to contaminate water supplies if the federal government did change the hours of service for truckers. A warning message was written on the letter; "Caution – Ricin – Poison". No one got sick and the facility was later declared clean.

In November 2003, a postal facility, handling mail for the White House, found a letter containing a vial of ricin. US Secret Service did not notify the FBI until six days later, which was considered remarkable since they were investigating the ricin letter in South Carolina at the time (see above). Since there was no threat against the public, the event was kept a secret until February 2004 when another ricin letter was discovered in Washington D.C. The FBI suspected that this letter, and the one found in South Carolina, were connected due to the poor quality of the ricin and that both letters were signed "Fallen Angel".

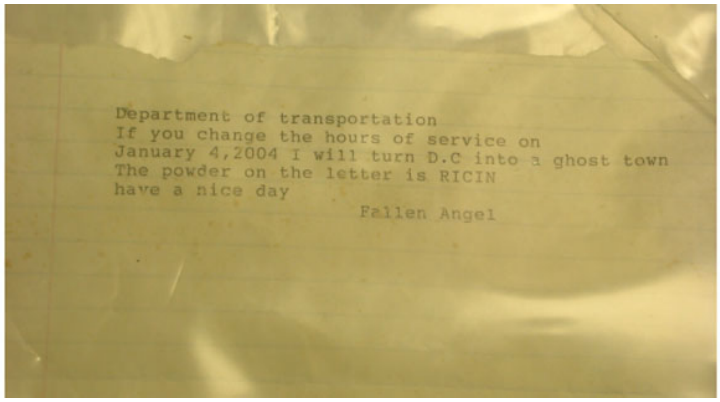

Figure 7. Ricin letter in Washington D.C. (Photo: FBI)

In February 2004, a powder was discovered in a letter opening machine in a mail room at a Senate Office Building in Washington D.C. The contents tested positive for ricin but it was determined that it would not present a health risk because of its low potency. Searches for a threatening note or an envelope were unsuccessful and it is doubtful if this event was a deliberate dispersion attempt. No illnesses were reported and two weeks after the findings, investigators raised the possibility that the positive test may have "been caused by paper byproducts".[47]

Availability of "ricin recipes" and dispersion in open sources

Data available in open sources describing ricin-making techniques is comprehensive. The so-called 'underground literature' offers books often cited in various extremists' circles; they all provide ricin- and abrin-making instructions. Information as regards manufacturing can also be found on the Internet. Many attempts are made to remove web sites containing this kind of information. To date, several have been removed, for example the web site exhibiting Hamas twenty-three page handbook *"Mujahahedeen Poisons Manual"*. On the very same site, results from an alleged test on rabbits using ricin are accounted for. According to the source, the Israel Defence Forces, IDF, the handbook was written in 1996. It contains facts on ricin and also details on how to manufacture poisons, chemicals, gases etc. to "be used in terrorist attacks against Israelis, Westerners and all others standing in the way of Islamic Jihad". According to the IDF, the author wrote in the manual that "the information in the manual is an abstract from material handed out at a course in poisoning", which he had attended together

[45] Brian Ross, *Posion plot. Ricin, cyanide found in Paris; U.S. facilities may have been targets,* 03/24/03
[46] CanWest Interactive, *Terrorist "poison" was wheat, barley,* 04/12/03
[47] *NBC: Investigators question ricin test result. Was positive reading in Senate office caused by paper byproducts?*, msnbc, 18/02/04, http://www.msnbc.com/id/4143594

with other terrorists, and that "the products have been tested on rabbits in order to assess their effectiveness"[48].

In 2000, in the Manchester home of an alleged al-Qaeda adherent, a manual of 180 pages was found letting Jihad warriors in on terrorism techniques. Moreover, one of the right extremists' web sites on the Internet offers an instruction on how various poisonous substances can be used for illegal purposes.

Also, on various web sites on the Internet, companies are selling what is called "rare products". One of the companies is said to sell ricin, curare, scopolamine and various snake venoms but has no web site of its own and, too, cannot be found in the "Yellow pages" on the Internet[49].

Other web sites offer production instructions, and there is one particular that offers, among other things, a CD containing instructions, dosage and miscellaneous information about various poisons and biological substances.

Acquisition and dispersion

Since ricin grows in many parts of the world, the beans are readily available. Its natural habitat in the US might explain why American groups and individuals are experimenting with production, and to a certain extent, with dispersion. Instructions regarding advanced dispersion of ricin cannot be found in so-called 'underground literature' or on the Internet. However, there are some tips to be found on how to poison people by dispersion in food and beverage or by injections. According to one of the tips in underground literature, an effective method of administering ricin percutaneously is mixing it with DMSO[50] and smearing the substance on doorhandles, steering wheels etc. On one hand, no one knows of such an attempt. On the other, it would most likely not work since proteins are not taken up by the skin, even though they are mixed with DMSO.

[48] Israel Defense Forces Home page, *Manual for poisons and chemical gases published on Hamas website,* 01/02/03, http://www.idf.il/newsite/english/0102-2.stm

[49] www.yellowpages.com

[50] DMSO, dimetylsulfoxid, categorized as an industrial solution, also used by veterinarians mainly on horses treating muscle soreness and damages to the soft tissues. DMSO very rapidly and effectively penetrates the skin and can probably transport certain low molecular substances to the bloodstream.

4. Effects

Toxicity

Ricin is classified as a "medium poisonous" toxin, for example 100-1000 times less toxic than botulinum toxin, but compared to for example sarin; ricin is approximately twenty times more toxic.

Such information is usually presented in the media and also on fact sheets on the Internet. Even though not untrue, it offers a simplified version of the truth. Animal and cell tests performed point to a complicated picture having variations depending on animal type, particle size, routes of exposure and individual variations, all joining in to make the verifying of "true" toxicity data more difficult. The toxicity data presented below are considered to come from reliable sources. Nevertheless, we would like to point out that calculations differing much from these have also been published.

Animal tests have established that ricin can not be spontaneously taken up by the skin; it must be injected, inhaled or ingested to be effective[51]. In the animal tests performed, the toxicity previously mentioned much depends on animal type and how the poison is administered; chickens and frogs are the least sensitive, horses the most sensitive.

In tests on mice, the LD_{50} following inhalation was decided to 3-5 μg/kg body weight, and following intravenous injection, 5 μg/kg. Intraperitoneal and subcutaneous injections gave approximately four times higher LD_{50} values. Following ingestion, a LD_{50} value of 20 mg/kg was measured. All tests were performed by the USAMRIID (the US Army Medical Research Institute of Infectious Diseases). The shortest time-span between administering and death is approx. 60 hours following inhalation, but according to other information, a more rapid progression of poisoning can be observed (see below).

When it comes to intake, in the cases in which poisoning of humans has been fatal, the victims are estimated to have consumed 12 and 20 seeds, respectively, namely 20 respective 35 mg of ricin, thus suggesting a very significant difference in sensitivity between humans and mice.

Symptoms

Since ricin can be taken up by all cell types, symptoms, course of illness and cause of death vary, taken into account the way in which the poison was administered.

What happens following intake is well documented due to the numerous reports on poisonings[52] [53]. In certain literature, the danger of ricin intoxication has been exaggerated. It is widely reported that "one seed is enough to kill a child" but this has not been verified. However, the lethality rate is very low when taken into account modern health care; in 1950-1997, 247 cases of ricin intoxications were reported; only one was fatal (in1950). However, the risk of ricin intoxication must not be underestimated, because as mentioned above, the information varies significantly as to an ingested lethal dose of ricin.

Following ingestion, the symptoms manifested appear after 4-12 hours; diarrhoea, nausea, vomiting and abdominal pains. In less severe cases, the symptoms of ricin poisoning include

[51] D.R. Franz and N.K. Jaax. Chapter 32: Ricin toxin In *Textbook of military medicine "Medical aspects of chemical and biological warfare"*. 1997, pp 631-42, Office of the Surgeon General, Department of the Army
[52] A. Rauber and J. Heard *Vet. Hum. Toxicol.* 1985;27:498-502 Castor bean toxicity re-examined; A new perspective
[53] P.J. Aplin and T. Eliseo *MJA* 1997;167:260-261 Ingestion of castor oil plant seeds

diarrhoea. In such cases, the patients usually recover without any medical care. In more severe cases, the symptoms are fever, thirst and headache, which can result in collapse and chock. After a lethal dose, death will follow in three to four days.

If ricin is injected, other symptoms follow such as fever, thirst and headache sometimes resulting in vascular collapse and chock. After a lethal dose, death will often follow in three to four days.

When it comes to the death of Georgi Markov[54], who was injected, the clinical picture is clear. The dose administered at that time is estimated to have been approx. 0,5 mg. The injection site hurt and became swollen almost immediately. A couple of hours later, Markov experienced weakness, palpitations and a fever set in. Gradually, complications set in, he experienced vascular collapse, went into chock and died early in the morning of the third day after the injury was inflicted. In this particular case, evidence of a potential use of ricin could not be produced. Anyway, it was concluded that the dose injected was very small, thus indicating a very potent toxin and, too, that the symptoms were similar to those observed in ricin intoxicated pigs. In addition to the Markov case, symptoms of poisoning have been studied in connection with clinical tests having as purpose to use ricin in the treatment of cancer. The symptoms picture concur well with that observed in the Markov case; influenza-like symptoms such as fatigue and muscle pain, sometimes followed by nausea and vomiting.

Following inhalation, only the symptoms originating from animal tests are verified. However, there are reports on allergic reactions in connection with handling of ricin seeds, but they are more likely to be the result of other components than of ricin. Since the symptoms following inhalation and ingestion concur well in animals and humans, it is likely that this also goes following inhalation. Animal tests on rats and monkeys give similar symptoms. In monkeys, the first symptoms observed were those of lacking appetite and less physical activity. After 8-24 hours, injuries are showing around the aerial ways and the mouth, and are then spread to the lungs thus increasing lung damages. Eventually, death will follow due to a collapsing circulation system. The progression of poisoning is somewhat more rapid than that following injection and ingestion. Following tests on monkeys all died within 48 hours.

Dispersion

Plausible dispersion methods for ricin would be those of poisoning food, water and beverage, or by using an aerosol or an injection. Since ricin is a protein, it is sensitive to its surrounding, for example to pH, salinity, and other possible substances interacting with it. Poisoning by injection proved to be effective, as in the assassination of Georgi Markov, but the method is for practical reasons only usable in certain operations.

In most animal tests, in which an aerosol is administered, it is difficult to decide whether a dry or a liquid aerosol is used. Since ricin is a protein it is depending on keeping its three dimensional structures in order to maintain the activity. When drying proteins, this structure is often partly destroyed. Even a liquid aerosol can affect the function of the protein in a negative fashion, among other things depending on the fact that the material coming in contact with air partly or entirely is destroyed. Following dispersion, the liquid is rapidly vaporized, bringing along a change in effectiveness of the toxin.

In terms of a large-scale dispersion, calculations show that approximately 8 tons of ricin is needed to kill half of the population within an area of 100 km², that is, approximately 80

[54] D.R. Franz and N.K. Jaax. Chapter 32: Ricin toxin In *Textbook of military medicine "Medical aspects of chemical and biological warfare".* 1997, pp. 631-42. Office of the Surgeon General, Department of the Army

mg/m². However, it is not certain in which way the ricin must be dispersed in order to accomplish this[55].

[55] D.R. Franz, chapter 30: Defense against toxin weapons In *Textbook of military medicine "Medical aspects of chemical and biological warfare"*. 1997, pp. 603-619. Office of the Surgeon General, Department of the Army

5. Protection

Detection and technical protection

Today there are a number of "detection tickets" for ricin. Along with technical innovations, detection instruments are becoming more and more similar to the analytical ones, as shown below, both intended to be of primary use in environmental sampling.

µChemLab™ has produced a so-called "lab-on-a-chip" using of concentration, micro-scaling and liquid and capillary chromatography in order to receive a separation profile that can be traced to known substances[56]**.** The system can also be used in order to more widely analyse low molecular chemical warfare agents and protein toxins, it is thus not based on a specific identification method (for example mass spectrometry or antibodies). By using this technique, nanomolecular concentrations (corresponds to approx. 1-10 µg/ml) can be detected (analysed).

Osborne Scientific Group (OSG) offers a "Ricin Toxin Rapid Identification Kit For Environmental Screening" having an analysis technique based on specific antibodies against ricin[57]. It is possible to identify 50 ng or 0.4 µg/ml ricin using this technique and the result can be read within a couple of minutes. This instrument is used by US governmental authorities.

Protection Mask 90 must eliminate 99.995 % of the particles in case of an aerosol dispersion. Since the ricin do not penetrate intact skin, no full protective clothing is needed.

Decontamination

For personal decontamination, a 0.5 % hypochlorite solution can be used. However, decontamination of intact skin is not in any way as critical as is exposure after, for example, chemical warfare agents such as nerve gas or mustard gas. When it comes to people having been exposed to anthrax spores, a comparison might be made regarding recommended procedures to be followed in the event of decontamination. In this case, personal decontamination with soap and water has been determined to be sufficient. There is not a single documented of dispersion of ricin indoors[58] and the possible need for decontamination has not yet been established, but in Washington D.C., some precautions were taken. The discovery of ricin prompted the closure of three Senate office buildings; Dirksen, Russell and Hart buildings. In the Dirksen building, where trace amounts of ricin were found, the ventilation system was shut down until after decontamination was accomplished. According to some sources, personal decontamination was made with soap and water. The carpets were steam cleaned and hard surfaces rubbed down with a mild chlorine-based solution. 1,300 air and surface samples were collected and tested, but "only a few" taken confirmed positive for ricin. After the decontamination, no additional traces were found.[59] As it looks, decontamination procedures recommended by the CDC[60] were used and apparently turned out to be successful.

---

[56] Fact sheet from µChemLab™:http://www.ca.sandia.gov/industry_partner/microchem/McCmLab.pdf
[57] Osborn Scientific Group home page: http://www.osborn-scientific.com/ricin.html
[58] The findings in 2004 in the letter opening machine in Washington D.C. are in this report not considered to be remnants after a deliberate dispersion, since it has not been verified as such.
[59] Matthew Cella, *Dirksen building reopens; Office tainted with poison closed for two more weeks*, The Washington Times, 10/02/04
[60] CDC, *Ricin Emergency Response Card (NIOSH) – ERC9009-86-3*, http://www.bt.cdc.gov/agent/ricin/erc9009-83-3.asp

Even though it might be relatively harmless to be in a room not yet decontaminated, this might cause psychological reactions since people most likely not are inclined to run the risk of being exposed.

Medical protection

So far, the treatment has been limited to treating the symptoms as extensively as possible. One reason for the poisonings reported lately having not been fatal, is that treatment offered today has been successful. Out of 751 poisonings registered, only 14 cases were fatal, and out of those, twelve occurred before 1930.

When it comes to ricin poisoning, the symptoms are delayed and non-specific, thus easily confused with those of other substances used intentionally (for example staphylococcus enterotoxin B and phosgene). Compared to many other protein toxins, ricin is also rapidly secreted from the body (in rats, approx. 90 % has disappeared after 24 hours[61]), indicating that a therapeutic treatment, in principal, must be administered in order to have an effect. 150 various chemicals have been tested in the treatment of ricin poisoning. Even though some substances inhibit ricin in an *in vitro* trial (among other things brefeldin-A and derivatives of D-galactose), none of those had any positive effect in animal tests[62], thus highlighting the need for an effective vaccine against ricin. The two vaccines having proven to be effective is a formalin treated toxoid and, too, the deglycosylated form of RTA (A-chain from ricin). However, they are not harmless since the formalin treatment is believed not to inactivate the toxin, and the RTA vaccine has proven to be able to give VLA (see Fields of Applications). Recently, reports have shown that both vaccines are easily improved. The effect of the formalin treated toxoid has been increased in that it is encapsulated in polymer micro spheres[63]. However, in the present report side-effects, if any, are not discussed. In an additional report, chain A on the ricin mutated in the active surface, resulting in a ceasing toxic effect of the protein, but also in the part of the protein contributing to VLS[64]. The effects of the two mutants tested are very satisfactory; no side-effects have been observed. Nevertheless, these tests have been performed on mice, but hopefully the following clinical trials will confirm the promising results.

[61] C. Ramsden, M. Drayson and E. Bell *Toxicology* 1989;55:161-171 The toxicity, distribution and excretion of ricin holotoxin in rats
[62] W. Thompson, J. Scovill and J. Pace *Natural toxins* 1995;3:360-377 Drugs that show protective effects from ricin toxicity in *in vitro* protein synthesis assays
[63] M. Kende, C. Yan, J. Hewetson, M.A. Frick, W.L. Rill and R. Tammariello *Vaccine* 2002;20:1681-1691 Oral immunization of mice with ricin toxoid vaccine encapsulated in polymeric microspheres against aerosol challenge
[64] J.E. Smallshaw, A. Firan, J.R. Fulmer, S.L. Ruback, V. Ghetie and E.S. Vitetta *Vaccine* 2002;20:3422-3427

6. Threat assessment

Ricin as a weapon

Ricin presents a threat since it has the necessary properties for being used in terrorist attacks. The source material, the castor bean, is easily accessed since the ricin bush can be found wild in several parts of the world. Moreover, it is a popular houseplant. The active substance is relatively inexpensive to produce, simple techniques are applied and no advanced technical skill is needed. It is highly toxic; however, it poses no direct threat when produced and stored. It stays intact for a long time at room temperature, indicating that the protein is relatively heat stable. There is no antidote, and the vaccines in use today are not completely harmless, factors of great importance from a terrorist's perspective.

Dispersion

Dispersion of ricin is probably the limiting factor in terms of usage of ricin. In this case, the technical skill necessary is found to exceed what is necessary for production.
In all reported cases, countries that had an offensive programme, trying to produce a ricin weapon, discontinued their programmes. Even if the reason was not only related to problems with dispersion techniques, it has most likely indicated to non-state actors that ricin is hard to disperse effectively outdoors over a vast area. Concerning non-state actors, in the cases when usage or confiscation of ricin has been reported, no one has tried to perform a medium- or large-scale attack but has directed them against single individuals.

Potential users

Even though the use of ricin as a (military) warfare agent compared to others is a less likely scenario, there are aspects worth noting. Many, but not every country has signed and ratified the CWC (or the BTWC) and is as such controlled by the CWC. Several of those countries that have not signed or ratified CWC or BTWC are believed to have, or have had, offensive biological or chemical research programs. It is probably easy to hide manufacture of ricin by pointing to the legal production of castor oil. Even though several countries are interested in ricin, there are no indications as to any country doing offensive research within the ricin area.

The extensive attention that ricin has received in the media for the past few years, may have contributed to the fact that it seems to have become attractive to terrorists and criminals. Those especially interested in ricin are at the moment (right-wing) extremists and al-Qaeda sympathizing groups. However, an analysis of events having occurred pin-points some interesting differences between the (right-wing) extremist groups in the US and al-Qaeda. In principle, the former have limited their activities to the production of ricin and on some cases also planned on using it. The knowledge about effective dispersion methods is probably limited within these circles and the possession of ricin is simply showing-off. On the other hand, al-Qaeda sympathizing groups may be the most likely ones to initiate an attack. Ricin can in this case be a "logical choice" since seeds – easily and legally – can be transported across borders or bought in place in order to produce the toxin. Dispersion would likely occur by contaminating food or by dispersing ricin in the air at a suitable location, for example in a subway. Even if the consequences wouldn't be so severe, the psychological effects could be extensive.

Psychological and other effects

Ricin has received extensive coverage in media the past few years which may have given some ideas to perpetrators, inspiring them to manufacture ricin. Even though the toxin hasn't been used in attacks, this is a worrying fact. A positive conclusion from the ricin findings in

the U.S. postal system is that the toxin seems to be in a crude form, too granular to make it possible to disperse it the air with the same effects as the Anthrax letters in 2001. An assumption that can be made is that the manufacture descriptions that circulate in open sources, like the Internet, do not result in a toxin suitable for dispersion in the air.
The psychological effects after dispersion (or attempts) are difficult to foresee and may depend on the dispersion method used. Ricin letters will most likely causing effects similar to those after the anthrax letters and also be compared with those. The difference is that there is no known antidote, unlike anthrax that can be prevented with antibiotics. This fact may cause another kind of fear among the public than the anthrax letters.
Considering the enormous consequences following dispersion of anthrax via letters, a threat using ricin letters could be costly and put heavy strain on society. The issue of contamination would be discussed but research is currently scarce. It is highly likely that people would insist on being decontaminated if they suspected themselves, or the premises, to be contaminated.

Vaccinations
An interesting observation can be made regarding the on-going vaccine research. Considering the fact that no country is assessed to carry out offensive ricin research at present, the threat of large-scale usage is considered to be low. Hence, the threat focuses on small or medium-sized terrorist attacks. In most cases, in which ricin poisoning has occurred, medical treatment focusing on symptoms turned out to be adequate. In this respect, it is somewhat surprising that large sums of money are spent on developing a vaccine. One question is whether the whole population should be given vaccine or just the key personnel.

Conclusive assessment
Due to its wide availability, simple production methods, lack of countermeasures and expected psychological effects, ricin is, and will be, of future interest in the eyes of terrorists and criminals. The shortcomings of the toxin; its relatively low toxicity and, too, general lack of knowledge about effective dispersion methods might result in ricin most likely not being part of governmental offensive programs or being used by non-governmental actors on a large scale. The possibilities of dispersing ricin in smaller areas and, too, the problems connected with decontamination have to be closely investigated in order to possibly reducing the prevalent fear of ricin as well as reducing the strong psychological effects provoked in the event of a ricin attack today.

Appendix 1. Incidents involving ricin

Below follows a resume of some of the events involving acquisition, possession or usage of ricin.

- In 1982, William Chanslor tried to purchase ricin from the author of the book "How to kill". He notified the police and Chanslor was arrested at an airport in Canada at which the deal was supposed to be settled. By using the ricin, he had planned to murder his wife who was paralyzed after having a stroke. Chanslor was sentenced to jail for three years and fined $5000.
- In 1983, two brothers in Massachusetts, USA, were arrested for manufacturing approx. 30 g of "almost pure ricin" which was stored in a film canister. It is unclear for whom the ricin was intended.
- In 1983, a nineteen year-old from Florida, attempted to murder his father with ricin. The teenager sent a friend to a company in order to purchase $200 worth of ricin. Reportedly, the buy was a success, but the friend had second thoughts and the ricin was replaced with water. The nineteen year-old was sentenced to three years in prison and an additional 200 hours a year of community service for a period of fifty years.
- In 1992, a group of right-wing extremists in the US manufactured 0.7 g of ricin. According to the plans, they intended to mix the ricin with a lotion and smear it on door handles. The prospective victims were all from the local authorities and policemen.
- In 1993, Thomas Lavy was caught by Canadian boarder police. 130 g of ricin and ricin seeds were found in his car. He stated that he intended to poison coyotes to keep them away from his chickens. Risking a life sentence, Lavy committed suicide in his prison cell before the trial.
- In 1995, Ray Mettetal's home was searched and also a rented safe-deposit box. In the latter, powdered ricin was found. At the trial, it was established that he had planned on murdering his former supervisor at the Vanderbilt University and the reason was that he had been excluded from an educational program in the mid 1980s. He confessed to having manufactured ricin but that he had not planned on using it. In 1998, Mettetal was sentenced to ten years in prison. At a new trial in 2001, he had his sentence reduced by 4 and half years.
- In 1995, a woman in Tennessee, USA, was arrested for producing ricin "enough to kill 4000 people". It is not established for whom the ricin was intended.
- In 1997, Thomas Leahy of Wisconsin, USA, was charged with possessing ricin, nicotine and dispersion equipment. In January 1998, he was sentenced to twelve year and seven months in prison without a chance of parole. According to the US Department of Justice, Leahy had been in possession of 0.7 g of ricin, enough to kill 125 people.
- In August 2001, 2 g of ricin was found in a man's home in Arkansas. He had plotted to kill his wife.
- Between 1998 and 2002, the US police on three occasions searched homes in which they found ricin seeds, equipment and production instructions.
- In 1999, James Kenneth Gluck sent a threatening letter to a judge at a Colorado court. It stated that he had armed himself the last two years and that he threatened to use ricin against the Jefferson County Justice Center in Golden, Colorado. Gluck was arrested and in November 1999, both ingredients for ricin and laboratory equipment

were found in his home. The alleged motive was a dispute concerning property that Gluck had owned in Colorado.

- In the beginning of January 2003, seven North African men were arrested, under the suspicion of producing ricin in an apartment in a London suburb, in which production equipment and "small amounts of ricin remnants" were found. Since no ricin was found they were suspected of having hid the toxin elsewhere.
- In October 2003, an envelope containing a threatening note and a sealed container was processed at a postal facility in Greenville, South Carolina. The letter, which was addressed to the Transportation Department and signed by the signature "Fallen Angel", said that large amounts of ricin would be used to contaminate water supplies if the federal government did change the hours of service for truckers. A warning message was written on the letter; "Caution – Ricin – Poison". No one got sick and the facility was declared clean.
- In November 2003, a postal facility, handling mail for the White House, found a letter containing a vial of ricin. US Secret Service did not notify the FBI until six days later, which was considered remarkable since they were investigating the ricin letter in South Carolina at the time (see above). Since there was no threat against the public, the event was kept a secret until February 2004 when another ricin letter was discovered in Washington D.C. The FBI suspected that this letter and the one found in South Carolina were connected due to the poor quality of the ricin and that both letters were signed "*Fallen Angel*".
- In January 2004, Astrid Tepatti and Ebony Wood were arrested with a bag of ricin. The two women were lovers and had plotted to kill Tepatti's husband for insurance money. The ricin, made by Tepatti and Wood was toxic, but not the poison in its most lethal form. It was found inside a vehicle the two women were travelling in near San Diego.
- In February 2004, a powder was discovered in a letter opening machine in a mail room at a Senate Office Building in Washington D.C. The contents tested positive for ricin but it was determined that it would not present a health risk because of its low potency. Searches for a threatening note or an envelope were unsuccessful and it is doubtful if this event was a deliberate dispersion attempt. No illnesses were reported and two weeks after the findings, investigators raised the possibility that the positive test may have "been caused by paper byproducts".
- In April 2004, Robert M. Alberg was arrested in Seattle for manufacturing ricin. Alberg had ordered castor seeds by mail from a seed company in New York, but the unusual amount of seeds, 5 pounds (more than 5,000 seeds) that he ordered in November 2003, made the employee contact the FBI. It was determined that the company should mail the seeds to Alberg in December 2003. For what purpose Alberg would use the ricin was unknown in June 2004.