

Safety Data Sheet

Ricin

Division of Safety
National Institutes
of Health



WARNING!

THIS COMPOUND IS EXTREMELY TOXIC. IT IS READILY ABSORBED BY VARIOUS BODY TISSUES THROUGH THE RESPIRATORY AND INTESTINAL TRACTS. IT MAY CAUSE SEVERE IRRITATION OF TISSUES (SKIN, EYES, MUCOUS MEMBRANES, AND LUNGS) AND INDUCE SENSITIVITY. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND HOT WATER. AVOID RUBBING OF SKIN OR INCREASING ITS TEMPERATURE.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNTS OF WARM ISOTONIC SALINE. FOR INGESTION, INDUCE VOMITING. DRINK MILK OR WATER. REFER FOR GASTRIC LAVAGE. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. ADMINISTER RESCUE BREATHING IF NECESSARY. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. USE ISOTONIC SALINE TO DISSOLVE COMPOUND. USE ABSORBENT PAPER TO MOP UP SPILL. WASH DOWN AREA WITH ALKALINE PERMANGANATE SOLUTION FOLLOWED BY SOAP AND WATER. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

Introductory Remarks

1. Ricin is often referred to in the literature as a "lectin." The original definition of a lectin was a plant seed protein which causes agglutination of erythrocytes. While this property was originally believed to be associated with ricin, it is now known that the hemagglutinating function of the ricinus seed (the natural source of ricin) resides in a hemagglutinin with properties similar to, but distinct and separable from, the toxic ricin.

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2. The ultimate identity of "pure" ricin has not been completely established. Older preparations, even though obtained in crystalline form, retained agglutinating and/or proteolytic properties. It is probable that the toxic molecule is "Ricin-D" (Ishiguro et al., 1964, 1971) and nearly all of the more recent investigations have been carried out on this material. An isotoxic ricin-E has also been described but not too well characterized. The Tai castor bean (large grain) appears to contain ricin-E only while the Japanese castor bean (small grain), the usual source of ricin, contains a mixture of D and E (Mise et al., 1977).

A. Background

Ricin is a toxic glycoprotein found in the beans of the castor plant (Ricinus communis) where it remains in the pomace after commercial separation of castor oil. The castor plant is also grown as an ornamental shrub, and poisoning has occurred (particularly in young children) due to chewing its leaves or beans. The beans, when chewed, are extremely toxic, one bean having been known to produce fatal poisoning (Dreisbach, 1983). Exposure to dust produced during the extraction of castor oil has resulted in toxic effects as well as skin rashes. Ricin is under investigation as a cytostatic drug in the treatment of some malignancies.

Earlier work on the chemical properties of ricin and its mechanism of action has been reviewed (Funatsu, 1972; Balint, 1974; Olsnes, 1976).

B. Chemical and Physical Data

1. Chemical Abstract No.: 9009-86-3
2. Synonyms: None.
3. Chemical structure and molecular weight: Ricin is a glycoprotein; its molecular weight has been reported variously between 55,000 and 64,000, with an average of 60,000. It consists of two chains (A and B) linked by a disulfide bond. Its structure has been represented schematically as follows, using the most recent pertinent literature reports (Yoshitake et al., 1978; Kimura and Funatsu, 1981):

<u>N-terminal</u>	<u>C-terminal</u>	<u>Names</u>	<u>Mol. wt.</u>	<u>No. of residues</u> <u>amino acids</u> <u>oligosac-</u> <u>charides</u>	
Ile	Ser	A-chain, Ile chain ("effectomer")	30,600	265	6
Ala	Phe	B-chain, Ala chain ("haptomer")	31,400	260	15

Diagram illustrating the structure of the A-chain and B-chain of Ricin. The A-chain (Ile chain, "effectomer") has an N-terminal Ile and a C-terminal Ser, with two S-S bonds between them. The B-chain (Ala chain, "haptomer") has an N-terminal Ala and a C-terminal Phe, with four (S-S)₄ bonds between them. The two chains are linked by two S-S bonds.

(Abbreviations for terminal amino acids: Ile = isoleucine; Ser = serine; Ala = alanine; Phe = phenylalanine). The above two references furnish the complete amino acid sequences of the A and B chains, respectively. The oligosaccharides consist of mannose and N-acetyl glucosamine chains attached to one (A chain) or two (B chain) asparagine residues. The role of these oligosaccharides has been discussed (Simeral et al., 1980). For toxicological information on ricin and its A and B chains see Section F.

4. Density: No data.
5. Absorption spectroscopy: $E_{280} = 1.18$ for ricin, 0.765 for A chain, 1.49 for B chain (Simeral et al., 1980). Minimum at 250 nm.
6. Isoelectric point: $7.1-7.3$ for ricin D, 7.5 for A chain, 4.8 for B chain (Olsnes, 1976),^A 8.8 for ricin E (Mise et al., 1977).
7. Volatility: May be considered negligible.
8. Solubility: Since ricin has the properties of an albumin it is soluble in dilute salt solutions.
9. Description: White powder or crystals.
10. Boiling point, melting point: Not applicable.

^AThese are considered to be the most reliable data. Note that previous publications list an isoelectric point of 5.9 for ricin D (Ishiguro et al., 1971). The difference may be related to the method of purification.

11. Stability: Solutions of ricin can be stored in frozen form even with repeated freezing and thawing. In the refrigerator these solutions are stable for several months on addition of 0.1 M galactose. Ricin is stable at acid pH (0.1 M acetic acid, 24 hr, room temperature) but is destroyed by alkali (0.1 M NaOH). The isolated chains are less stable. Intact ricin is resistant to attack by proteolytic enzymes (trypsin, chymotrypsin, pepsin, Pronase) while the isolated chains are sensitive to these enzymes (Olsnes et al., 1975). "Nagarse"^A partially releases about 30 amino acid residues from the Ile chain, without loss of toxicity (Funatsu et al., 1970).
12. Chemical reactivity: Ricin produces positive tests with the usual protein reagents (biuret, ninhydrin, etc.). Reductive methylation lowers toxicity only slightly and has no effect on protein synthesis inhibition (Sanvig et al., 1978). Treatment of ricin with 2-mercaptoethanol results in cleavage of the disulfide linkage between the A and B chain. This reaction is reversed by dialysis. Toxicity is destroyed on treatment with KMnO₄ (30 mg/L, 20 min), iodine (16 mg/L, 30 min), and other oxidants such as ozone, hydrogen peroxide, chlorine, and bromine (Carmichael, 1929; Delga, 1954).
13. Flash point: Not applicable.
14. Autoignition temperature: Not applicable.
15. Explosive limits in air: Not applicable.

C. Fire, Explosion, and Reactivity Hazard Data

1. As a protein, ricin is inactivated under conditions of fire. The main hazards would be from the formation of dusts which could be toxic on inhalation or skin exposure. Therefore, fire-fighting personnel should wear complete protective clothing and air-supplied respirators with full face masks.
2. Flammability is likely to be low.
3. Conditions contributing to instability (and detoxification) are high temperatures and alkali.
4. No hazardous decomposition products are known.

D. Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The NIH

^AThe identity of this material could not be established; it is assumed that it is a commercial preparation of proteolytic enzymes.

Guidelines should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving ricin.

It should be emphasized that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

1. Chemical inactivation: Methods have been reported (Sigma Chemical Co., 1980).
2. Decontamination: Turn off equipment that could be affected by ricin or the materials used for cleanup. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Use absorbent paper to mop up spill. Wipe off surfaces with alkaline permanganate, then wash with copious quantities of water. Glassware should be rinsed in a hood with alkaline permanganate, followed by soap and water. Animal cages should be washed with water.
3. Disposal: It may be possible to decontaminate waste streams containing ricin before disposal. For details, see Sigma Chemical Co., 1980. No waste streams containing ricin shall be disposed of in sinks or general refuse. Surplus ricin or chemical waste streams contaminated with ricin shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing ricin shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing ricin shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with ricin shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing ricin shall be handled in accordance with the NIH radioactive waste disposal system.
4. Storage: Store solid ricin and its solutions in dark-colored, tightly closed containers, in a freezer. Store in sealed ampoules or in bottles with caps with polyethylene cone liners

inside a sealed secondary container. Store working quantities of ricin and its solutions in an explosion-safe refrigerator in the work area.

E. Monitoring and Measurement Procedures Including Direct Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: No data.
2. Analysis: There are no specific chemical methods available for the analysis of ricin since the protein molecule does not carry any signatory chemical groupings. A radioimmunoassay, useful for identification of poisoning with ricin and for monitoring patients under treatment has been published. Blood concentrations down to 50-100 pg/ml blood can be determined by this method (Godal et al., 1981).

F. Biological Effects (Animal and Human)

1. Absorption: Ricin is absorbed and produces toxic effects by ingestion, parenteral injection, and inhalation as dust. It is also a potent irritant of eyes, nose, and skin but it is not known whether systemic effects are produced via any of these routes.
2. Distribution: Ricin labeled with ^{125}I (labeling occurs in both chains and does not affect toxicity) and administered intravenously or intraperitoneally is distributed, in order of decreasing concentrations, to spleen, kidney, heart, liver, and thymus. Simultaneous administration of lactose with ricin considerably decreases the binding of ricin to tissue proteins. At least up to 5 hours after injection the radioactivity is found mostly in the form of intact ricin. Tissue radioactivity had almost entirely disappeared 10-20 hours after administration (Fodstad et al., 1976).
3. Metabolism and excretion: The mechanism of metabolism of ricin has not been investigated but presumably consists of proteolysis. No radioactivity due to parenteral administration of ^{125}I -labeled ricin is found in the feces; urinary excretion is fairly rapid, and mostly in the form of trichloroacetic acid-soluble products (Fodstad et al., 1976).
4. Toxic effects: The intraperitoneal and intravenous LD50 of ricin in mice and rats has been variably reported to be between 2.8 and 8 $\mu\text{g}/\text{kg}$ (Olsnes and Pihl, 1973; Derenzini et al., 1976; Fodstad et al., 1977; Fodstad and Pihl, 1978).^A A review report

^APublished LD50s by these authors are usually given in terms of ng or $\mu\text{g}/\text{animal}$. Their data have been converted here to $\mu\text{g}/\text{kg}$ for readier intercomparison.

(Olsnes and Pihl, 1978) states, without documentation, that "Lethal doses of...ricin are about 1 ug toxin/kg body weight in the mouse, rat, and dog, whereas the rabbit is about 10 times more sensitive."

Parenteral injection of doses slightly above the LD50 into rats produces primarily liver necrosis. The effect is much more directed towards the Kupffer cells than the parenchymal cells. This is in line with other findings that Kupffer cells show a much higher sensitivity to inhibition of protein synthesis by ricin, and the uptake of ¹²⁵I-labeled ricin by these cells is considerably higher than by parenchymal cells (Derenzini et al., 1976; Skilleter et al., 1981). Necrosis of, and inhibition of protein synthesis by, spleen is also marked. Neurological effects have also been demonstrated (Harper et al., 1980; Wiley et al., 1982). In man, ricin intoxication is characterized by a long latent period, which may last for hours or days depending on dose (the same applies to administration of ricin to experimental animals), before onset of symptoms. Ingestion results in severe gastroenteritis which is often hemorrhagic. Later symptoms are: drowsiness, coma, occasional convulsions, peripheral circulatory collapse, oliguria which may lead to uremia and death (Gosselin et al., 1976; Dreisbach, 1983). Exposure of eyes, nose or throat to dusts of ricin (or of castor bean pomace) may result in local inflammation. Allergic reactions after such exposures have been noted but it is not clear whether they are due to ricin or a naturally concomitant separate allergen.

The mechanism of toxic action of ricin consists of a potent inhibition of protein synthesis, due to interference with the incorporation of amino acids at the site of the 60S ribosomal subunit. This has been most clearly demonstrated in cell cultures and cell-free systems (for reviews see Olsnes, 1976; Olsnes and Pihl, 1978). In exerting this effect in cell cultures, and to produce toxicity in animals, the intact ricin molecule is required. Protein synthesis is inhibited by the A chain only if its addition to cell cultures is preceded by that of the B chain no more than 90 minutes previously (Houston, 1982). In cell-free systems, by contrast, the A chain is fully as active as the intact ricin while the B chain does not contribute to the activity. On the basis of these and other findings it is believed that the B chain ("haptomer") is responsible for binding the ricin molecule to the cell surface, allowing the A chain ("effectomer") to penetrate the cell interior and to exert its toxic effect. The binding to the cell surface appears to be via a galactose moiety on the cell, since each B chain contains one binding site for galactose and lactose, and the presence of lactose in a cell incubation medium prevents the toxic action of ricin.

5. Carcinogenic effects: No carcinogenic effects of ricin have been reported. On the contrary, ricin has a strong cytostatic effect against transplanted malignancies in mice (Lin et al., 1970, 1971; Fodstad et al., 1977; Fodstad and Pihl, 1978). The mechanism is presumably the same as that described above for normal cells, i.e., inhibition of protein synthesis in the malignant cell. Ricin appears to show selectivity for malignant cells, and in addition has the advantage over other cytostatics in not depressing the level of white cells and having only a moderate effect on erythropoiesis and thrombopoiesis (Fodstad et al., 1977).
6. Mutagenic and teratogenic effects: None have been reported.

G. Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and hot water. Avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with copious quantities of warm isotonic saline followed by running water for at least 15 minutes. Obtain ophthalmological evaluation.
2. Ingestion: Drink plenty of water or milk. Induce vomiting. Refer for gastric lavage.
3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.
4. Refer to physician at once. Consider treatment for pulmonary irritation.

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