

Status of Developing TEEL values for Biotoxins

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Biotoxins:

- Biotoxins are produced from terrestrial or marine animals, plants, fungi, or bacteria
- Protein toxins
- Non-protein toxins
- Possible warfare agents
- Most potent biotoxins are neurotoxins



Source: National Oceanic and Atmospheric Administration
http://www.noaanews.noaa.gov/stories2009/20090917_ohi_ohab.html

Historical Use of Biotoxins in Warfare

Date	Event
Mid 1300s	Mongols catapult plague-infected corpses over the walls of Crimea
Late 1400s	Spanish try to contaminate wine with blood from leprosy patients
Mid 1700s	Smallpox-infected blankets were distributed to the Indians
1941-42	Aerial bombs and cannon shells tested for biological warfare - anthrax spores at Gruinard Island, Scotland
2001	Letters contaminated with anthrax spores were distributed in the U.S.

From: *Toxicology* (2005) 214:167-181

Where We Began

1. Ricin
2. Abrin
3. Botulinum toxins
4. SEB
5. Tetrodotoxin
6. Tetanus toxin
7. Fusariotoxin, T-2
8. Conotoxins
9. Cholera toxin
10. Shigella

Biotoxins

- Ricin/Abrin



- Tetrodotoxin



- Conotoxins



Biotoxins

- Botulinum toxins



- Tetanus toxin



- Shigella



INITIAL APPROACH



Broad Search

- Google Scholar
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Find articles with **all of the words** Results p
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without the words
 where my words occur

Author Return articles written by
 e.g., "PJ Hayes" or McCarthy

Publication Return articles published in
 e.g., J Biol Chem or Nature

Date Return articles published between -
 e.g., 1996

Collections **Articles and patents**

Search articles in all subject areas (include patents).

Search only articles in the following subject areas:

Google scholar [Advanced Scholar Search](#) [My Citations](#)

Scholar Results 1 - 10 of about 18,800. (0.13 sec)

[Dependence of ricin toxicity on translocation of the toxin A-chain from the endoplasmic reticulum to the cytosol](#) [HTML] from jbc.org

[cytosol](#)

J Wesche, A Rapak... - Journal of Biological Chemistry, 1999 - ASBMB

Abstract Ricin acts by translocating to the cytosol the enzymatically active toxin A-chain, which inactivates ribosomes. Retrograde intracellular transport and translocation of ricin was studied under conditions that alter the sensitivity of cells to the toxin. For this purpose ...

[Cited by 162](#) - [Related articles](#) - [BL Direct](#) - [All 6 versions](#)

[Identification and characterization of a monoclonal antibody that neutralizes ricin toxicity in vitro and in vivo](#)

[vivo](#)

PV LEMLEY, P AMANATIDES... - Hybridoma, 1994 - online.liebertpub.com

ABSTRACT We wanted to identify and characterize MAbs with specificity for the toxic lectin ricin, which could serve as detection reagents in elucidating mechanisms and tissue distribution. Neutralizing MAbs could be developed into immunotherapeutics to reverse

Refined Search

- Changed search phrase
- Over 2,000 hits

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Ricin
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e.g., J Biol Chem or Nature

e.g., 1996

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Scholar Articles and patents anytime include citations [Create email alert](#) Results 1 - 10 of about **2,020**. (0.11 sec)

[Toxicity, distribution and elimination of the cancerostatic lectins abrin and ricin after parenteral injection into mice.](#) [\[PDF\] from nih.gov](#)
[Find It @ HS/HSL](#)

O Fodstad, S Olsnes... - British journal of cancer, 1976 - ncbi.nlm.nih.gov
... Received 12 April 1976 Accepted 3 June 1976 Summary.-The survival time of mice after iv injection of the cancerostatic lectins, abrin and **ricin**, was recorded. The **LD50** dose was found to be 10-13 ng and 55-65 ng per mouse for abrin and ricin, respectively. ...
[Cited by 77](#) - [Related articles](#) - [Find It @ UMCP](#) - [FindIt @ UMBC](#) - [All 8 versions](#)

[Feasibility of immunodiagnostic devices for the detection of ricin, amanitin, and T-2 toxin in food](#) [\[PDF\] from tetracore.com](#)

EAE Garber, RM Eppley, ME Stack... - Journal of Food ..., 2005 - ingentaconnect.com
... **Ricin** (oral **LD50** 1 mg/kg), amanitin ... Despite a low toxicity level (oral **LD50** es- timated at 1 mg/kg of body weight (8)), the notoriety of **ricin** in the media has led its classification as a potential threat agent (3). **Ricin** is a heterodimeric protein consisting of two subunits (13, 15). ...

Refined Search

- Changed search phrase again
- Only 146 hits, but most files are not relevant

The screenshot shows a Google Scholar search interface. The search bar contains the text "Ricin \"LC50\"", which is circled in red. To the right of the search bar is a "Search" button and a link to "Advanced Scholar Search". Below the search bar, the "Scholar" logo is followed by filters: "Articles and patents", "anytime", "include citations", and "Create email alert". On the right side of this bar, the text "Results 31 - 40 of about 146. (0.25 sec)" is circled in red. The search results list includes:

- [Biosynthetic origin of oxygen atoms in DIMBOA from maize: NMR studies with 18O2](#)
E Glawischnig, W Eisenreich, A Bacher, M Frey... - *Phytochemistry*, 1997 - Elsevier
... Frs F17 to F26 were pooled (6.8 g; BST LC50 0.12 Ag ml) and further fractionated on a second open column (id = 5 cm, packed with 600 g of 60 200 mesh silica gel) using a hexane acetone gradient ... (!) Formation of the intramolecular formaldehyde acetals of javo] ricin [9]: a ...
[Cited by 18](#) - [Related articles](#) - [Find It @ HS/HSL](#) - [BL Direct](#) - [All 5 versions](#)
- [Cancer cell inhibitors and method](#)
BV Lipps... - *US Patent 5,565,431, 1996* - [Google Patents](#)
... 15, 1996 Sheet 8 of 11 Panel/Cell Line LDG10LC50 "FT 0.79 0.50 0.82 m LC50 Leukemia CCRF-CEM HL-60(TB) K-562 MOLT-4 "MI-8226 Non-Small Cell Lung Cancer A549/ATCC EK W HOP-62 HOP-92 NCI-H226 NCI-H23 NCI-H460 0.87 0.94 0.78 0.78 1.00 0.79 0.81 Colon ...
[Cited by 4](#) - [Related articles](#) - [All 2 versions](#)
- [\[PDF\] CLONING AND EXPRESSION OF cDNA ENCODING RIBOSOME INACTIVATING PROTEINS](#) [\[PDF\] from uasd.edu](#)
S ABBAS - 2007 - [etd.uasd.edu](#)
... The LC50 of ricin to the silkworm larvae at third instar was much lower than that of

ACCEPTED APPROACH



TEEL Development for Biotoxins

- Found in Handbook Version 2.1 (draft)
- Begin with the same hierarchy of data used to develop TEEL values for other chemicals
- Start with exposure limits
- Look for applicable toxicity data

TEEL Development for Biotoxins

- Toxicity data search
- LC50, LD50, etc.
 - HSDB
 - SAX
 - RTECS
- Google Scholar Search
 - Confirm/verify/reject

“...If after these data sources are searched and little or no data are found, a literature search using Google Scholarto find journals and other appropriate publications. Preference is given to articles that report data as toxicity endpoints (e.g., LC50 or LD50)...”

“...If after these sources are searched and little or no data are found...”

Handbook Version 2.1

TEEL Development for Biotoxins

- Human data are preferred but may not be applicable to Operational Emergency
 - Therapeutic uses of many biotoxins, such as BOTOX®
 - Novel advances using them for medical applications/treatments (e.g., pain, cancer)
 - Used in suicide attempts
 - Accidental poisonings
- Mouse units?
- Advances beyond medicine – pesticides

Lessons Learned

- Much data available, but most is not relevant
- The direction of research constantly changing
- For our purposes, older research is more applicable
- Biotoxins are complicated
 - Multiple serotypes
 - Multiple polypeptides/proteins
 - Different functions for the toxin mode of action
 - Cell binding, target molecule binding

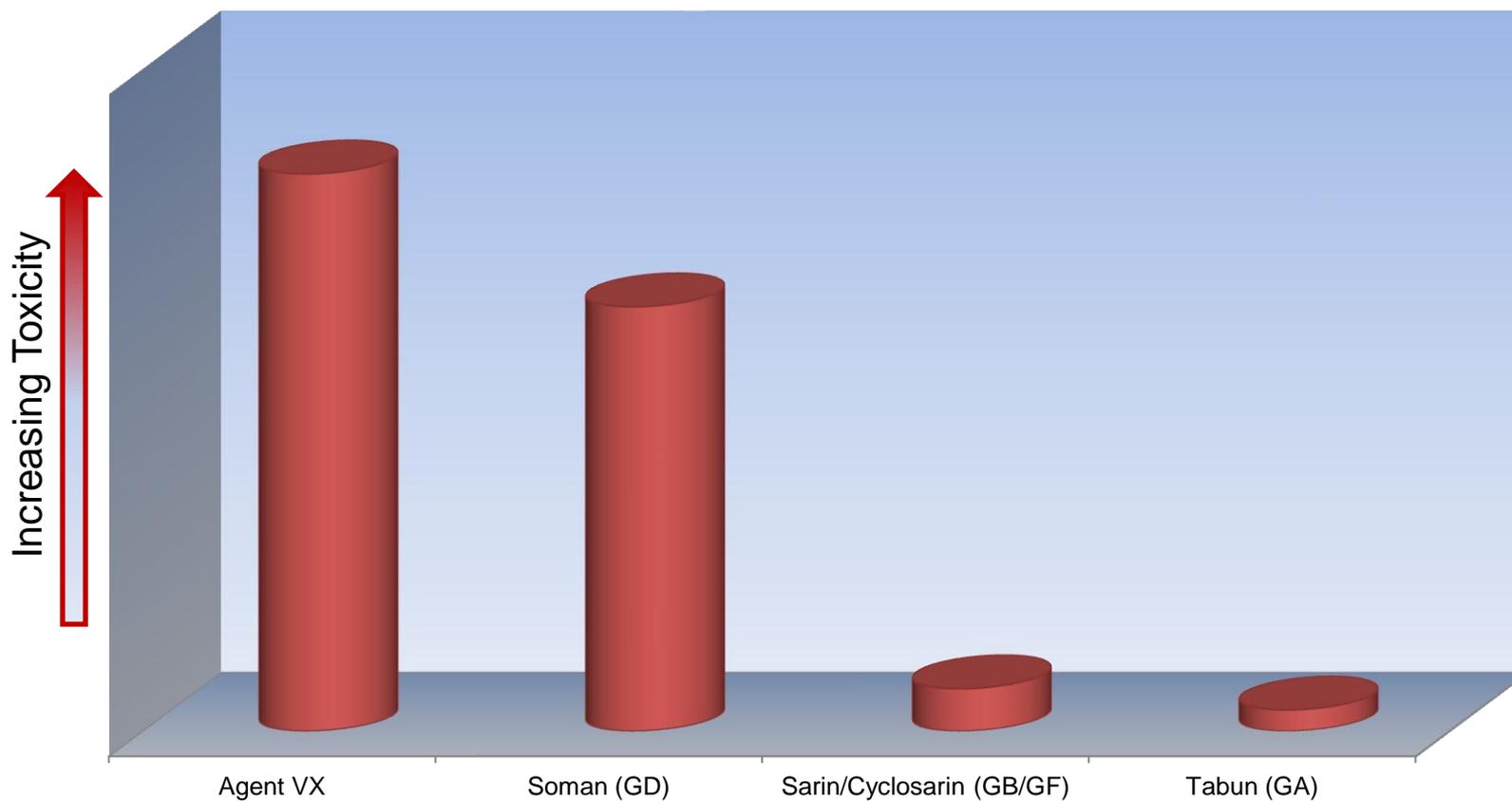
RESULTS



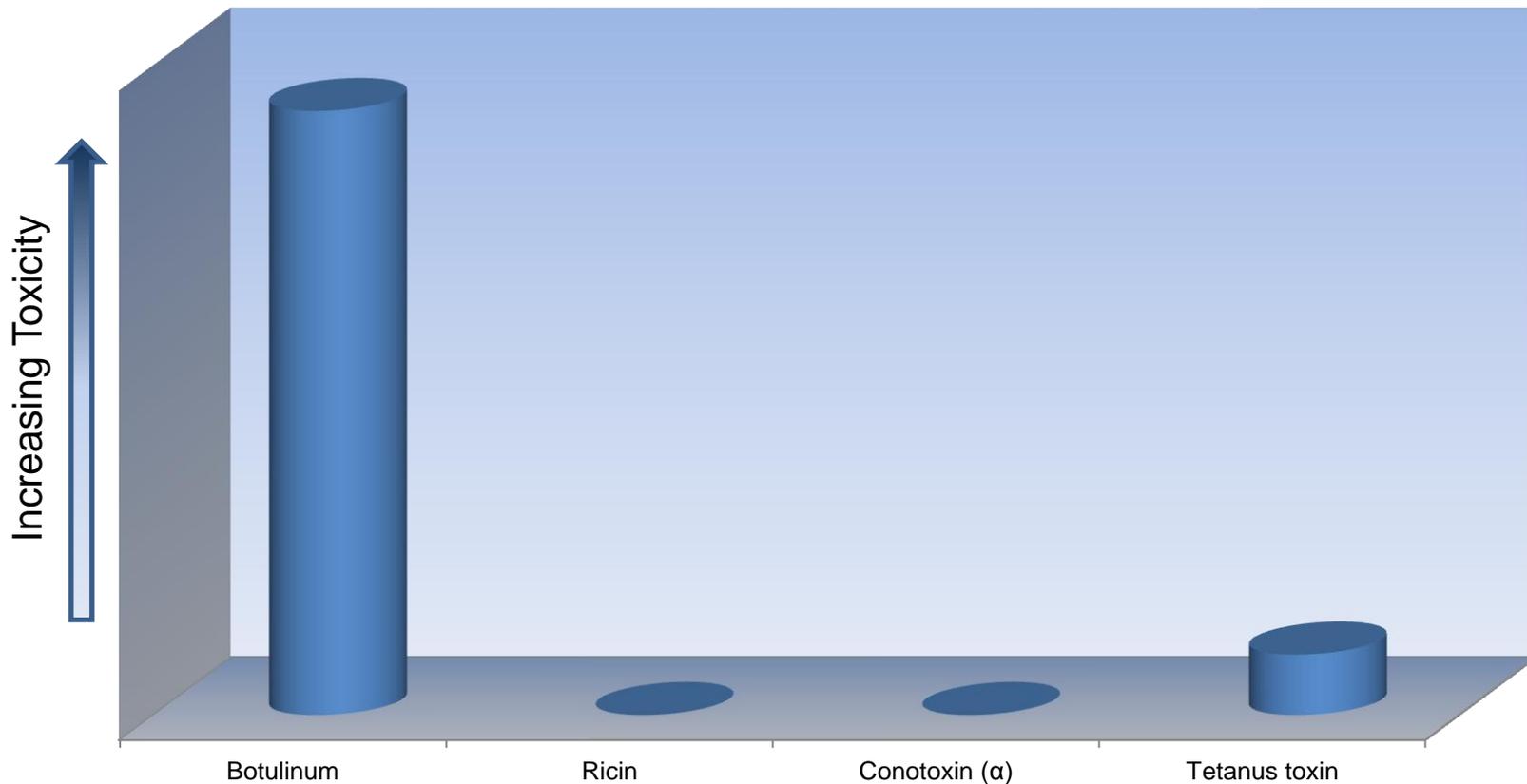
Preliminary Results (in mg/m³)

Biological Toxin	TEEL-1	TEEL-2	TEEL-3
Abrin	1.30E-07	1.40E-06	8.70E-06
Botulinum toxin - General	6.60E-10	7.20E-09	4.30E-08
Cholera toxin	8.50E-04	0.0094	0.056
Conotoxin (α)	1.60E-05	1.80E-04	0.0011
Ricin	2.00E-04	6.50E-04	6.50E-04
Saxitoxin	5.10E-05	5.60E-04	0.0033
Shiga toxin	8.20E-07	9.00E-06	5.40E-05
SEB	3.60E-06	4.00E-05	2.40E-04
T-2 Fusariotoxin	2.80E-04	0.0031	0.093
Tetanus toxin	6.60E-09	7.20E-08	4.30E-07
Tetrodotoxin	7.10E-04	0.0078	0.047

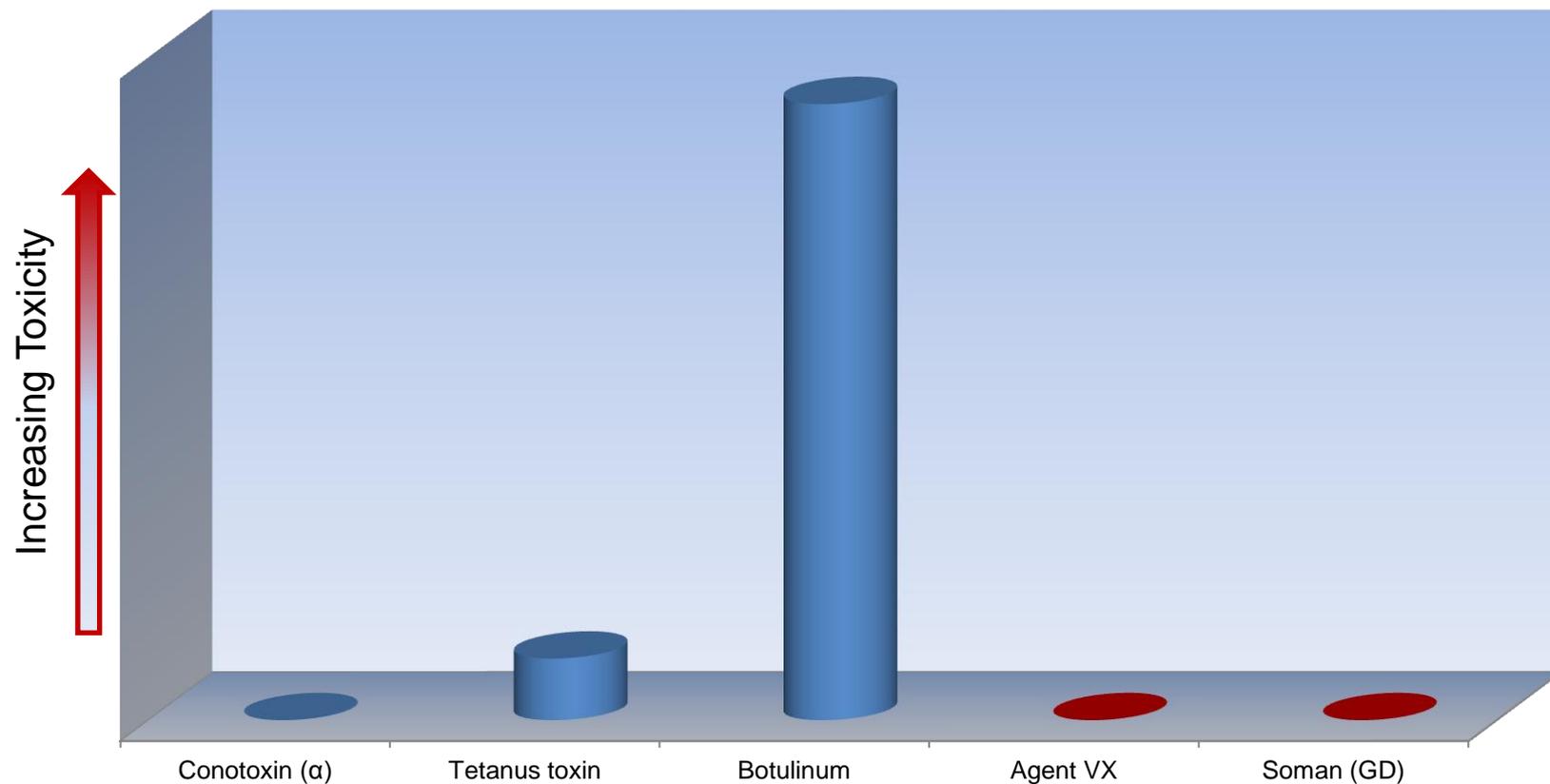
Relative Toxicities of Chemicals with the Smallest AEGL Values



Relative Toxicities of Four Biotoxins



Relative Toxicities Compared



So we ask:

- Why are biotoxins so toxic?
 - Most are proteins with specific target molecules
 - Highly selective for that specific target molecule
- Why is this of practical significance?
 - Small amounts can incapacitate an entire army (SEB)
 - Small amounts can be lethal (botulinum, ricin)

Future Plans

- Bio Safety Working Group joint meeting
- Submit draft TEELs to NA-41 for consideration
- Additional biotoxins remain
 - Some have published exposure limits
 - Confirm data/data sources with BSWG

QUESTIONS?

