**Cryoprotectant toxicity**

**Cryoprotectant toxicity** is the dose-dependent chemical injury that is produced by exposing biological cells, tissues or organs to chemicals that reduce ice formation ([cryoprotectants](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryoprotectant)). Cryoprotectant toxicity should be distinguished from other mechanisms of cryopreservation injury such as [chilling injury](http://www.evidencebasedcryonics.org/wiki/index.php?title=Chilling_injury) (injury produced by low temperatures as such), [cold shock](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cold_shock) (injury produced by rapid cooling or a large temperature drop), and osmotic damage. Cryoprotectant toxicity itself can again be divided into *general cryoprotectant toxicity* and *specific cryoprotectant toxicity*. General cryoprotectant toxicity involves the concentration (water substitution) effects of cryoprotectants and specific cryoprotectant toxicity involves the effects of specific cryoprotectants on cellular viability. Cryoprotectant toxicity is the single biggest obstacle to reversible low-temperature cryopreservation of biological cells, tissues and organs. Eliminating cryoprotectant toxicity is one of the most important research objectives in low temperature organ preservation and [cryonics](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryonics).

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**[**[**edit**](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryoprotectant_toxicity&action=edit&section=1)**] Cryoprotectants**

* [2,3−Butanediol](http://www.evidencebasedcryonics.org/wiki/index.php?title=2,3%E2%88%92Butanediol)
* [Dimethylformamide](http://www.evidencebasedcryonics.org/wiki/index.php?title=Dimethylformamide)
* [Dimethyl sulfoxide (DMSO)](http://www.evidencebasedcryonics.org/wiki/index.php?title=Dimethyl_sulfoxide)
* [Ethylene glycol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Ethylene_glycol)
* [Formamide](http://www.evidencebasedcryonics.org/wiki/index.php?title=Formamide)
* [Glycerol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Glycerol)
* [Methanol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Methanol)
* [N-Methylformamide](http://www.evidencebasedcryonics.org/wiki/index.php?title=N-Methylformamide)
* [Propylene glycol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Propylene_glycol)
* [Trehalose](http://www.evidencebasedcryonics.org/wiki/index.php?title=Trehalose)

**[**[**edit**](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryoprotectant_toxicity&action=edit&section=2)**] Assessment of cryoprotectant toxicity**

Cryoprotectant toxicity can be assessed by a number of different means. Different means of assessment may result in different results. The capacity of cells to replicate is a simiple assessment, but is not applicable to gametes or non-proliferative tissues. Fertility or motility is often used to assess viability in gametes. A number of [viability assays](http://www.evidencebasedcryonics.org/wiki/index.php?title=Viability_assay) can be used to assess the functional capacity of cells and tissues.

**[**[**edit**](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryoprotectant_toxicity&action=edit&section=3)**] Temperature-dependence of cryoprotectant toxicity**

Cryoprotectants are generally less toxic at lower temperatures, although the mechanism for this effect may vary. [DMSO](http://www.evidencebasedcryonics.org/wiki/index.php?title=DMSO) shows very pronounced reduction of toxicity with temperature reduction {[CRYOBIOLOGY; Wang,X; 55(1):60-65 (2007)](http://www.ncbi.nlm.nih.gov/pubmed/17618999)}. DMSO is more hydrophobic at higher temperatures, which can cause more membrane dehydration {[BIOPHYSICAL JOURNAL; Sum,AK; 85(6):3636-3645 (2003)](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1303668/)}. DMSO oxidation of sulfhydryl groups is probably lessened by lower temperature {[BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS; Snow,JT; 64(1):441-447 (1975)](http://www.ncbi.nlm.nih.gov/pubmed/238511)}.

**[**[**edit**](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryoprotectant_toxicity&action=edit&section=4)**] Cryoprotectant toxicity specific to cells, tissues and organs**

Cryoprotectants or their metabolites can cause chemical damage to specific tissues. [Ethylene glycol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Ethylene_glycol) is metabolized to oxalic acid, which can form damaging calcium oxalate crystals in the kidney {[TOXICOLOGY LETTERS; Guo,C; 192(3):365-367 (2010)](http://www.ncbi.nlm.nih.gov/pubmed/19931368)}. [Glycerol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Glycerol) can also cause kidney damage, but by increasing nitric oxide release {[RENAL FAILURE; Chandler,V; 28(2):161-169 (2006)](http://www.ncbi.nlm.nih.gov/pubmed/16538975)}. [Methanol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Methanol) is metabolized to formic acid which can cause blindness by destruction of the optic nerve {[LIFE SCIENCES; Tephly,TR; 48(11):1031-1041 (1991)](http://www.ncbi.nlm.nih.gov/pubmed/1997785)}.

Glycerol is the least toxic cryoprotectant for kidney slices, and is much less toxic than [DMSO](http://www.evidencebasedcryonics.org/wiki/index.php?title=DMSO) for spermatazoa {[POULTRY SCIENCE; Tselutin,K; 78(4):586-590 (1999)](http://ps.fass.org/cgi/reprint/78/4/586)}. But glycerol is much more toxic than other cryoprotectants to flounder embryos {[THERIOGENOLOGY; Chen,SL; 63(4):1207-1219 (2005)](http://www.ncbi.nlm.nih.gov/pubmed/15710204)} and *Escherichia coli* bacteria {[CRYOBIOLOGY; Markarian,SA; 49(1):1-9 (2004)](http://www.ncbi.nlm.nih.gov/pubmed/15265712)}.

**[**[**edit**](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryoprotectant_toxicity&action=edit&section=5)**] Toxicity neutralization by mixing cryoprotectants**

Cryoprotectants can neutralize the toxicity of other cryoprotectants. The heat-release-on-mixture of [DMSO](http://www.evidencebasedcryonics.org/wiki/index.php?title=DMSO) with other cryoprotectants correlates with the order of effectiveness of reducing DMSO toxicity by those cryoprotectants. Specifically, [formamide](http://www.evidencebasedcryonics.org/wiki/index.php?title=Formamide) has greater heat-release-on-mixing and greater effect on DMSO toxicity reduction than does [ethylene glycol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Ethylene_glycol), and the effect of ethylene glycol is greater than for [N-Methylformamide](http://www.evidencebasedcryonics.org/wiki/index.php?title=N-Methylformamide) {[CRYOBIOLOGY; Fahy,GM; 24(3):196-213 (1987)](http://www.ncbi.nlm.nih.gov/pubmed/3595164)}. More recently, it has been claimed that DMSO reduces formamide toxicity, but not vice-versa {[CRYOBIOLOGY; Fahy,GM; 60(3 Suppl):S45-53 (2010)](http://www.ncbi.nlm.nih.gov/pubmed/19501081)}, but adding DMSO to 15% and 20% formamide raises kidney slice viability to nearly 100%, which would not be possible with DMSO alone.

Addition of the non-toxic cryoprotectants sucrose or [trehalose](http://www.evidencebasedcryonics.org/wiki/index.php?title=Trehalose) drastically reduces the toxicity of the less toxic isomers of [2,3−butanediol](http://www.evidencebasedcryonics.org/wiki/index.php?title=2,3%E2%88%92butanediol) on red blood cells {[[CRYOBIOLOGY; Bouton,P; 31(4):367-373 (1994)](http://www.ncbi.nlm.nih.gov/pubmed/7924394)}. Trehalose also reduces the toxicity of DMSO on oyster embryos {[AQUATIC LIVING RESOURCES; Chao,N; 7(2):99-104 (1994)](http://www.alr-journal.org/index.php?option=article&access=standard&Itemid=129&url=/articles/alr/pdf/1994/02/alr94206.pdf)}. For flounder embryos, there is a significant reduction in the toxicity of [glycerol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Glycerol), [DMSO](http://www.evidencebasedcryonics.org/wiki/index.php?title=DMSO), [propylene glycol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Propylene_glycol), and [ethylene glycol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Ethylene_glycol) by addition of 5% [methanol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Methanol) {[THERIOGENOLOGY; Zhang,YZ; 63(3): 763-773 (2005)](http://www.ncbi.nlm.nih.gov/pubmed/15629795)}.

**[**[**edit**](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryoprotectant_toxicity&action=edit&section=6)**] Mechanisms of cryoprotectant toxicity**

Protein denaturation is often thought to be a mechanism of cryoprotectant toxicity {[CRYOBIOLOGY; Arakawa,T; 27:401-415 (1990)](http://dx.doi.org/10.1016/0011-2240%2890%2990017-X)}. The enzyme thermolysin can be inactivated by [dimethylformamide](http://www.evidencebasedcryonics.org/wiki/index.php?title=Dimethylformamide) and, to a lesser extent, by [DMSO](http://www.evidencebasedcryonics.org/wiki/index.php?title=DMSO) — an effect that is opposed by [glycerol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Glycerol) and [trehalose](http://www.evidencebasedcryonics.org/wiki/index.php?title=Trehalose) {[JOURNAL OF BIOTECHNOLOGY; Pazhang,M; 127(1):45-53 (2006)](http://www.ncbi.nlm.nih.gov/pubmed/16860424)}. But randomly selected purified proteins required much higher concentrations for denaturation than was associated with cryoprotectant toxicity {[CRYOBIOLOGY; Fahy,GM; 27(3):247-268 (1990)](http://www.ncbi.nlm.nih.gov/pubmed/2199153)}.

A study of erythocyte hemolysis by cryoprotectants showed that the degree of hemolysis was almost entirely dependent upon the shape-change induced in the erythrocytes. The smaller the difference between the hydrophobicity of the solution and the hydrophobicity of the membrane, the greater the extent of hemolysis and change in shape. The toxicity neutralization of [formamide](http://www.evidencebasedcryonics.org/wiki/index.php?title=Formamide) and [DMSO](http://www.evidencebasedcryonics.org/wiki/index.php?title=DMSO) could be due to the opposing effects of the two cryoprotectants on solution hydrophobicity {[BIOCHEMICA ET BIOPHYSICA ACTA; Bakaltcheva,IB; 1280(1):73-80 (1996)](http://www.ncbi.nlm.nih.gov/pubmed/8634318)}.

It has been postulated that the hydrogen-bonding strength of cryoprotectants is the cause of both cryoprotectant effectiveness and cryoprotectant toxicity (although sugars are non-toxic). The metric **qv\*** has been used to quantify toxicity as a function of molecular polar groups at concentration of cryoprotectant needed to [vitrify](http://www.evidencebasedcryonics.org/wiki/index.php?title=Vitrify) kidney slices, where

**qv\*** = (**MW**)/(**MPG**)

for **MW** = moles of water

and **MPG** = moles of cryoprotectant polar groups

at a cooling rate of 10ºC per minute {[CRYOBIOLOGY; Fahy,GM; 48(1):22-35 (2004)](http://www.21cm.com/pdfs/improved_vitrification.pdf)}. For example, **MPG** of [glycerol](http://www.evidencebasedcryonics.org/wiki/index.php?title=Glycerol) is 3 polar groups and **MPG** of [DMSO](http://www.evidencebasedcryonics.org/wiki/index.php?title=DMSO) is 1 polar group. It is suggested that the toxicity associated with a high **qv\*** is due to the reduction in water availability for hydration of membranes and macromolecules.

(See also [Sample calculations of **qv\***](http://www.benbest.com/cryonics/viable.html#calcs))

**[**[**edit**](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryoprotectant_toxicity&action=edit&section=7)**] The importance of cryoprotectant toxicity**

If cryoprotectant toxicity could be eliminated or significantly reduced, then it could be possible to preserve organs for transplant at cryogenic temperatures — which would mean those organs could be available for virtually unlimited time periods (rather than having a short shelf life, as is currently the case). This could excite interest by large, well-capitalized corporations to invest in organ preservation technologies which could benefit [cryonics](http://www.evidencebasedcryonics.org/wiki/index.php?title=Cryonics). It would also give greater credibility to cryonics and could result in better preservation of cryonics patients. The better cryonics patients are preserved, the less question there will be about the ability of future technology to reanimate them — and the greater the chance that future technology will indeed be able to reanimate them.