

NIH Toxic Chemical Reduction Initiative

The NIH Toxic Chemical Reduction Initiative (TCRI) started in 2007 to promote pollution prevention and waste reduction at the NIH and in response to [Executive Order 13693](#) (former [EO 13514](#) and others also supported this effort) The NIH TCRI encourages the replacement of toxic chemicals generated as waste in biomedical research with less toxic and safe alternatives. It also recommends the use of automated equipment that operate efficiently, protects health of operators and meets requirements of environmental protection by using less toxic reagents.

The accomplishment of this goal was spearheaded by the Division of Environmental Protection (DEP) in consortium with the NIH Environmental Management System (NEMS) Sustainable Laboratory Practices Working Group (SLPWG) that initially identified and developed a master list of about 200 NIH Toxic Chemicals to replace or reduce from NIH's research. See [Toxic Chemical Master List](#).

The toxic chemicals were selected based on [four criteria](#) including large volume of waste generation (at NIH), health and safety risks, official reduction mandates and the availability of feasible reduction methods. For management purposes, the selected chemicals were organized into the following 3 groups:

- Group I: NIH selected toxic chemicals (prioritized due to available alternatives)
 - Group II: NIH selected toxic chemicals (predominately solvents used in high volume)
 - Group III: NIH selected toxic chemicals (discontinued and or generated at lower volumes)
- I. **Group I NIH Selected Toxic Chemicals** were prioritized chemicals with already available alternatives in the market. They consists of ethidium bromide, phenol and chloroform, phosphoric acid, chromic acid, picric acid, ethylene oxide, mercury and its compounds, as listed in the table below with health and safety risks, uses and alternatives for NIH researchers:

Group I NIH Selected Toxic Chemicals			
Toxic Chemical	Health and Safety Risk	Uses	Alternative*
Ethidium Bromide	<ul style="list-style-type: none"> • Highly toxic, and combustible • Potent mutagen • Irritant to skin, eyes, mouth and nose 	<ul style="list-style-type: none"> a. DNA stain b. Gel electrophoresis 	<ul style="list-style-type: none"> ▪ SYBR Safe (a) RQ1ⁱ ▪ SYBR® Gold (a,b) RQ1ⁱⁱ ▪ SYBR® Green I (a,b) RQ1ⁱⁱ ▪ Gold View™ (a,b) RQ1ⁱⁱ ▪ Methylene blue (a) RQ1ⁱⁱⁱ ▪ Azure A (a) RQ1ⁱⁱⁱ ▪ Brilliant cresyl blue (a) RQ1ⁱⁱⁱ ▪ Gel Red (a) RQ1ⁱ ▪ GreenGlo (a) RQ3^{iv} ▪ Agilent Bioanalyzer chip (b) RQ3^v ▪ EzVISION™ (a) RQ3^{vi} ▪ Gel Green™ (a) RQ3^{vii} ▪ E Gel
Phenol/Chloroform	<p>Phenol:</p> <ul style="list-style-type: none"> • Highly toxic, corrosive and combustible • Liver toxin • Irritant to eyes, skin and mucous membranes 	<ul style="list-style-type: none"> a. Traditional DNA extraction 	<ul style="list-style-type: none"> ▪ New DNA extraction kits using polycarbonate filters (a) RQ1^{viii} Ref 1 ▪ Magnetic microspheres (urea-formaldehyde and silica microspheres) (a) RQ1^{ix} Ref 1 ▪ Qiagen kits (a) RQ1^x Ref1 ▪ Machery-Nagel (MN) (a) RQ1^x Ref1 ▪ Trizol ▪ Magnetic microspheres MACS

Group I NIH Selected Toxic Chemicals

Toxic Chemical	Health and Safety Risk	Uses	Alternative*
	<ul style="list-style-type: none"> • Toxic and acts on CNS to cause death Chloroform: • Toxic • Carcinogen • Mutagen • Teratogen • Irritant to eyes, skin and respiratory track 		Separation columns <ul style="list-style-type: none"> ▪ EZI advanced Qiagen kits ▪ Pure gene kits ▪ Maxwell from Promega ▪ Hi Pure Plasmid Filter Maxi Prep Kit ▪ ZR Plasmid Miniprep-Classical Kit ▪ ZymoClean Gel Recovery Kit ▪ Fast Plasmid Mini kit ▪ EZ DNA methylation ▪ RNeasy Mini kit (RNA extraction) ▪ NucleoSpin RNA11(RNA extraction) ▪ PEG, and simple salts
Phosphoric acid	<ul style="list-style-type: none"> • Corrosive and causes burns • Irritant to eyes, skin and upper respiratory tract 	a. Cage cleaning b. Descaling c. HPLC d. Reagent by chemists e. Adjusting the pH of phosphate buffers	<ul style="list-style-type: none"> ▪ Formic acid (a, b) RQ3^{xi} ▪ Citric acid, Urid (a, b) RQ3^{xii} ▪ Bio-Strip 200 (a, b) RQ3^{xiii} ▪ UPLC
Chromic acid	<ul style="list-style-type: none"> • Corrosive • Irritant to eyes and skin • Carcinogen • Mutagen 	a. Chromic acid baths	<ul style="list-style-type: none"> ▪ Alconox (a) RQ1^{xiv} ▪ Citranox, Liqui-Nox, No Chromix, Contrad 70 (a) RQ1^{xv} ▪ Potassium hydroxide, potassium permanganate with sulphuric acid, Hydrochloric acid (a) RQ1^{xiv} ▪ Nitric acid (a) RQ1^{xvi, xvii} ▪ Alconox powder detergent (a) RQ3^{xviii} ▪ Citranox liquid acid cleaner & detergent(a) RQ3^{xviii} ▪ Detergent 8 low foaming (a) RQ3^{xviii} ▪ Luminox cleaner (a) RQ3^{xviii} ▪ Solujet low foaming liquid detergent (a) RQ3^{xviii} ▪ Teragajet powder detergent (a) RQ3^{xviii} ▪ Terg-A-Zyme Powder Detergent (a) RQ3^{xviii} ▪ Base baths ▪ Disposable labware
Picric acid	<ul style="list-style-type: none"> • Corrosive • Irritant to eyes and skin 	a. Fixative	<ul style="list-style-type: none"> ▪ Modified Davidson's Fixative (a) RQ1^{xix} ▪ Davidson fixative (Ethanol, acetic acid, formalin) (a) RQ1^{xx}
Ethylene oxide	<ul style="list-style-type: none"> • Extremely flammable gas • Irritant to eyes, skin and respiratory tract • Carcinogen 	a. Sterilization b. Reagent in chemical reactions – Used by chemists	<ul style="list-style-type: none"> ▪ Steam (a) RQ1^{xxi} ▪ Radiation (Gamma & electron-beam), plasma, microwave radiation (a) RQ1^{xxii} ▪ Vapor Phase Hydrogen peroxide (VPHP), peracetic acid (a) RQ1^{xxiii} ▪ Hydrogen Peroxide Gas Plasma (a) RQ1^{xxiv, xxv} ▪ Disposable sterile items

Group I NIH Selected Toxic Chemicals			
Toxic Chemical	Health and Safety Risk	Uses	Alternative*
Mercury	<ul style="list-style-type: none"> Corrosive and causes burns Irritant to eyes, skin and respiratory tract Carcinogen 	a. Single vial fixative for concentration, permanent stain, EIA, IFA and PCR procedures	<ul style="list-style-type: none"> Z-7 Fixative (a) RQ1^{xxvi} SAF Fixative (Sodium Acetate Acetic Acid Formalin) (a) RQ1^{xxvii} PROTO-Fix CLR Parasitology Fixative (a) RQ3^{xxviii} Zenkers Fixative Modified (a) RQ3^{xxix} Z-5 Fixative
Mercury cont'd	<ul style="list-style-type: none"> Mutagen Reproductive toxic Persistent bioaccumulative toxic (PBT) Marine pollutant 	a. B-5 mercury based fixatives and other fixatives	<ul style="list-style-type: none"> AZF (Acetic Zinc Formalin) Fixative (a) RQ1^{xxx, xxxi} B-Plus Fixative(TM) (a) RQ3^{xxxii} B5 Fixative Modified (a) RQ3^{xxxiii} Histo-Fix (a) RQ3^{xxxiv} Zenkers Fixative Modified Shandon Zinc Formal-Fixx™ (a)
Mercury cont'd		a. Hematoxylin stain	<ul style="list-style-type: none"> Harris Hematoxylin
Mercury Compounds	<ul style="list-style-type: none"> Irritant to eyes, skin and respiratory tract Carcinogen 	a. Parasitology – permanent staining, concentration, EIA, and ELISA procedures	<ul style="list-style-type: none"> SAF Fixative (Sodium Acetate, Acetic Acid Formalin) (a) RQ1^{xxvii} Eco-Fix (a) RQ1^{xxxv} Proto-Fix (a) RQ1^{xxxvi}
Mercury Compounds	<ul style="list-style-type: none"> Irritant Carcinogen Mutagen 	a. Thimerosal b. Mercuric chloride PVA for permanent staining	<ul style="list-style-type: none"> Methyl paraben, Propyl paraben, Thymol (a) RQ1^{xxxvii} Zinc-PVA parasitology transport vials

*Nomenclature legend for alternative reference literature using Reference Quality (RQ) index: RQ1 (govt. issued/academia/peer-reviewed), RQ2 (trade journal/book/newsprint/non-profit), RQ3 (vendor/industry). Disclaimer: NIH does not endorse any specific vendor.

II. **Group II NIH Selected Toxic Chemicals** are predominately solvents used in research and constitutes the top ten most generated wastes at NIH. They include methanol, acetonitrile, xylene, hexane, dichloromethane, formaldehyde, dimethylformamide, methyl pyrrolidone, trichloroacetic acid, carbon tetrachloride, trichloroethane and perchloric acid. Listed below is a table of Group II NIH Selected Toxic Chemicals with health and safety risks, uses and alternatives for NIH researchers

Group II NIH Selected Toxic Chemicals			
Toxic Chemical	Health and Safety Risk	Use	Alternatives ^(*)
Methanol	<ul style="list-style-type: none"> Flammable liquid and vapor Moderate eye irritant, moderate skin irritant and fatal or cause blindness if swallowed Neurotoxic, Vision 	a. Clearing agents in histology b. In Situ c. Western blotting d. Karl Fischer Titration e. Organic synthesis by chemists	<ul style="list-style-type: none"> Gas-expanded Liquids (e) RQ1^{xxxviii} Ionic Liquids (e) RQ1^{xxxix} Isopropanol (c) RQ1^{xxxix} Microwave Irradiation (e) RQ1^{xl} Semi-Dry and Dry Apparatus (c) RQ1^{xli} Heat-mediated (c) RQ1^{xlii} Histo-Clear (National Diagnostics, RA Lamb) (a)RQ1^{xliii} Clear-Rite 3™ (a)RQ1^{xliii}

Group II NIH Selected Toxic Chemicals

Toxic Chemical	Health and Safety Risk	Use	Alternatives ^(*)
	impairment and CNS depression		<ul style="list-style-type: none"> ▪ Histoclear XTM/ Shandon Xylene Substitute (a) RQ1^{xliii} ▪ CitriSolv (Fisher) (a) RQ1^{xliii} ▪ Invitrogen kits (c) RQ1^{xliiv} ▪ Ethanol (d, e) RQ3^{xlv} ▪ Mediclear IITM ▪ Pro-Par Clearant ▪ Trans-Blot TURBO Kits (Bio Rad Company) ▪ Protein Simple WES (c) ▪ Solvent recycling systems
Acetonitrile	<ul style="list-style-type: none"> ▪ Flammable liquid and vapor ▪ Eye irritant as lachrymator, mild skin irritant, may be fatal with complications if ingested. 	<ol style="list-style-type: none"> a. HPLC b. Solvent c. Organic synthesis d. Oligo and peptide synthesis e. Mass spectrometry 	<ul style="list-style-type: none"> ▪ Acetone (e) RQ1^{xlvi} ▪ Ethanol (a) RQ1^{xlvii} ▪ Use capillary columns (b) RQ1^{xlviii} ▪ Reduce flow rates ▪ Purchase oligonucleotides and peptides from Integrated DNA Technologies. Other specialty oligos are ordered from Applied Biosystems (now under the umbrella of Life Technologies/ThermoFisher Scientific).
Xylene	<ul style="list-style-type: none"> ▪ Flammable liquid and vapor ▪ Eye irritant, skin irritant and respiratory tract irritant. ▪ CNS, kidneys, liver & blood impairment. ▪ Mutagen on chronic exposure. 	<ol style="list-style-type: none"> a. Ehrlich Indole Procedure b. Radioactive tracer studies (liquid scintillation cocktails) c. Staining-histology/cytology d. Reaction solvent by chemists 	<ul style="list-style-type: none"> ▪ Sub-X, Bio-Clear (c) RQ1^{xlix} ▪ Micro-Clear (Micron Environmental Industries/VA) (a) RQ1^l ▪ Hemo-De (a, c) RQ1^{xliii,l,li} ▪ Citrisolve (c) RQ1^{xliii} ▪ Clear-Rite 3 (Richard-Allan), Pro-Par Clearant (Anatech Ltd), Formula 83 (CBG Biotech), Clear Advantage, XS-3 Xylene Substitute & SlideBrite (c) RQ1^{xliii} ▪ Histosolve (Shandon) EMS Xylene Substitute (a, c) RQ1^{xlix,li} ▪ Linear alkyl benzene (b) RQ1^{lii} ▪ Bio-Safe II – p to 25% aqueous samples with high efficiency (b) RQ1^{liii}, RQ3^{liv} ▪ Bio-Safe NA (highest efficiency for organic samples and dry filters) (b) RQ1^{liii}, RQ3^{liv} ▪ Econo-Safe II-economical cocktail for routine applications, including wipe tests (b) RQ3^{liv} ▪ Phenylxylylethane, Di-isopropyl-naphthalene (b) ▪ Solvent recycling systems

Group II NIH Selected Toxic Chemicals

Toxic Chemical	Health and Safety Risk	Use	Alternatives ^(*)
Dimethyl formamide	<ul style="list-style-type: none"> ▪ Flammable liquid and vapor ▪ Irritant to skin, eyes, GIT and respiratory system 	<ol style="list-style-type: none"> a. DNA extraction b. Bioactive compounds extraction from botanicals c. Used as a reagent in chemical synthesis 	<ul style="list-style-type: none"> ▪ Commercial DNA extraction kits (See Phenol/chloroform alternatives in Group I). ▪ Polyethylene glycol (a) RQ1^{lv, lvi} ▪ Microwave-assisted extraction and ultra-pure water as extraction solvent for extracting bioactive compounds from botanicals (b) RQ1^{lvii}
Dichloromethane	<ul style="list-style-type: none"> ▪ Toxic liquid ▪ Severe eye irritant, skin and GIT irritant, CNS impairment at high concentration. ▪ Carcinogen 	<ol style="list-style-type: none"> a. Traditional DNA extraction b. General reaction solvent by chemist c. Chromatography solvent by chemists d. Extraction of natural products e. Clearing agent f. Alternative to DCM for Biphasic Reactions <ul style="list-style-type: none"> ○ Alkylation ○ Amidation ○ Nucleophilic Substitution Reaction 	<ul style="list-style-type: none"> ▪ New DNA extraction kits. See Phenol/Chloroform alternatives in Group I. ▪ Diethoxymethane (b) RQ1^{lviii} ▪ Benzotrifluoride (trifluorotoluene) (c) RQ1^{lix} ▪ Ethyl acetate/ethanol (c) RQ3^{lx} ▪ Super critical CO2/Biobased liquids, glycerol, and liquid polymers (f) RQ1^{lxi} ▪ Ionic liquids (b) RQ1^{lxii} ▪ D-Limonene (d) RQ1^{lxiii} ▪ 2-Methyltetrahydrofuran (f) RQ1^{lxiv}
Hexane	<ul style="list-style-type: none"> ▪ Flammable liquid and vapor ▪ Irritant and neurotoxin 	<ol style="list-style-type: none"> a. Tissue Fixative b. Reaction solvent c. Chromatography solvent 	<ul style="list-style-type: none"> ▪ Cetyltrimethylammonium chloride (CTAC) (a) RQ1^{lxv} ▪ Ionic liquids (b) RQ1^{lxvi} ▪ Solvent Recycling System ▪ Several green fixatives as stated for Xylene and Formaldehyde in Group II.
Formaldehyde	<ul style="list-style-type: none"> ▪ Flammable liquid and vapor ▪ Irritant to skin & eye (conjunctivitis & corneal damage), GIT and CNS ▪ Carcinogen 	<ol style="list-style-type: none"> a. Tissue Processing (fixative) b. Biomolecule preservation 	<ul style="list-style-type: none"> ▪ Glyo-Fixx[®], FineFix[®], Cell-block[®], Green-Fix[®] (a) RQ1^{lxix} ▪ PAGA (Polyethylenglycol, ethyl Alcohol, Glycerol, Acetic acid), two zinc-based fixatives (ZBF, Z7), and commercially-available alternatives (RCL2 and CellBlock) (a) RQ1^{lxxii} ▪ Streck Cell Preservative and Cell-Free DNA BCT (b) RQ1^{lxxiii} ▪ Water ▪ Solvent Recycling System (Formalin)
Trichloroacetic acid	<ul style="list-style-type: none"> ▪ Corrosive white solid ▪ Burns to eye & skin, severe damage to GIT 	<ol style="list-style-type: none"> a. In situ hybridization reaction b. Sonication/homogenization after it has been stained 	<ul style="list-style-type: none"> ▪ Purchase oligonucleotides and peptides from Integrated DNA Technologies. Other specialty oligos are ordered from Applied Biosystems (now under the

Group II NIH Selected Toxic Chemicals			
Toxic Chemical	Health and Safety Risk	Use	Alternatives ^(*)
	& respiratory tract.	with Evans blue for certain biochemical reactions c. Precipitating proteins d. Oligonucleotide synthesis	umbrella of Life Technologies/ThermoFisher Scientific).
N-Methyl-2-Pyrrolidone	▪ Combustible & Irritant	a. Reaction solvent by Chemists	▪ Solventless or Solvent-Free Reactions
Carbon Tetrachloride	▪ Carcinogen ▪ Toxic by inhalation	a. Reagent solvent by Chemists	▪ Cyclohexane, Methyl Acetate, Benzotrifluoride (BTF) (a) ^{lxix} ▪ Solventless or Solvent-Free Reactions
1,1,1-trichloroethane	▪ Irritant	a. Reagent Solvent by Chemists b. Holding acrylamide gels	▪ Water ▪ Volatile Methyl Siloxanes (a) RQ1 ^{lxix}

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III. **Group III NIH Selected Toxic Chemicals** consists of the remaining chemicals in the master list and are generated at fewer volumes due to minimal usage on biomedical research or are no longer used at NIH. These chemicals include:

Group III NIH Selected Toxic Chemicals
▪ Heavy metals (lead, chromium, cadmium, silver, barium, and selenium)
▪ Persistent, Bioaccumulative and Toxic Chemicals seldom used at NIH like: Chlordane, Heptachlor, Methoxychlor, Dioxins, Furans, Dioxin compounds, and Polychlorinated biphenyls hardly seen on light ballasts.
▪ Other EPRCA-TRI chemicals like: Antrazine, Hydrogen Fluoride, Benz(a)Anthracene, Naphthalene, Fluoranthene, Pentachlorophenol, Copper, etc
▪ Rest of listed chemicals: Pyrene, Naphthalene, Hydrogen Bromide, Benzo(a)Pyrene, Fluorene, Hexachloroethane, Endosulfan, etc

The Toxic Chemical Reduction Initiatives Program had seen a gradual reduction of waste generated due to appropriate support by the NIH intramural community, the use of less toxic alternatives and new innovative techniques that use less or no toxic chemicals. See Toxic Chemicals Waste Data for [FY2007 to FY2015](#).

Another important Toxic Chemical Reduction Initiative implemented at NIH is the [Mercury Amnesty Program](#) which ensures compliance and revitalizes the NIH's (Mad as a Hatter) Mercury-Free Campaign.

To contribute to NIH's toxic chemical reduction efforts and share information on new alternatives and innovations in your lab, please complete the Toxic Chemical Reduction Initiative [Survey](#). Annual outreach programs to promote toxic chemical reduction initiative are conducted during DEP's hazardous regulation inspection and at the NIH Green Lab Fairs and Research Festivals to gather additional information about new alternatives. It is pertinent that reduction goals be achieved without causing negative adverse impacts on the NIH biomedical research mission.

Currently, the NIH/DEP is recovering xylene (including ethanol and formalin) which is one of the Group II solvents from specific waste streams by distillation with a solvent recycler for reuse in histology research. Most chemicals have shown a steady reduction over the past years and NIH researchers need to contribute dramatically towards reduction of Group II chemicals in order to achieve a significant impact.

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