SPECIFIC METHODS FOR THE DESTRUCTION OF HAZARDOUS CHEMICALS IN THE LABORATORY

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ACETONITRILE

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Acetonitrile (CH_3CN; MeCN)[1] is a colorless, poisonous, and flammable liquid. It is a teratogen and a skin and a severe eye irritant (bp 81.6 °C) [75-05-8].[2] Acetonitrile is widely used as an high-performance liquid chromatography (HPLC) mobile phase component. It has a number of uses in the laboratory and is used industrially. It is miscible with water, ethanol, and organic solvents. It is immiscible with petroleum ether.

Principles of Destruction and Decontamination

Acetonitrile may be hydrolyzed to acetic acid and ammonia by NaOH in an aqueous solution.[3] The amount of acetonitrile remaining is <0.025% after 15 days at room temperature or 2 h at 80 °C. See also the Organic Nitriles monograph for a reductive approach to the destruction of acetonitrile.

Destruction Procedure

As ammonia is evolved, all operations should be carried out in a properly functioning chemical fume hood. Dilute the acetonitrile solution with water, if necessary, until the

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concentration of acetonitrile is 10% or less. For each 1 mol of acetonitrile that is present, add 2.5 mol of NaOH in the form of a 10 M NaOH solution. If the acetonitrile solution is acidic (e.g., from HPLC buffer salts), it may be necessary to add more NaOH to maintain a strongly basic solution. Stir at room temperature (25 °C) for 15 days or at 70–80 °C for 2 h, neutralize, check for completeness of destruction, and discard the solution.

Analytical Procedures

Acetonitrile may be determined by gas chromatography (GC) using a 1.8-m × 2-mm i.d. column packed with Porapak Q.[3] The carrier gas was nitrogen flowing at 25 mL/min. A flame ionization detector was used, the oven temperature was 180 °C, and the approximate retention time of acetonitrile was 3 min. The limit of detection was 0.025%. A headspace technique was used. The sample was diluted at least 10-fold with 1 M pH 8 phosphate buffer, and a 10 mL aliquot was heated at 55 °C for at least 30 min in a 100 mL flask fitted with a rubber septum. A 1 mL portion of the headspace vapor was injected onto the column.

Related Compounds

This procedure is specific for acetonitrile.

Alternatives

Bearing in mind the shortages of acetonitrile in 2008–2009,[4] it might be desirable to design chromatographic procedures to minimize the consumption of acetonitrile, for example, using a slower flow rate or columns with smaller internal diameters.[5] Devices are available that allow mobile phase between peaks to be recycled. When peaks elute the mobile phase is diverted to waste and when peaks are not present the mobile phase is returned to the mobile phase reservoir, reusing the mobile phase is generally not recommended.[6] However, with appropriate quality control, it might be possible, in some cases, to reuse the mobile phase for less-critical applications. For some separations, using electrochemical detection recycling the mobile phase may even be advantageous.[7] Repeated passage through the guard cell (placed before the injector) will remove the impurities from the mobile phase. Recycled mobile phase should be filtered to remove any particulates that may form. Also, repeated sparging with helium may lead to the loss of the more volatile component.[8] HPLC-grade acetonitrile can be recovered using spinning-band distillation.[9] HPLC mobile phases frequently pose a major hazardous waste problem for laboratories and some thought should be given to their disposal when planning analytical procedures. In particular, chlorinated solvents should be avoided.[10]

Lei et al. caution that trifluoroacetic acid in acetonitrile-containing mobile phases can cause partial hydrolysis to acetamide which can interfere with low-wavelength detection.[11]

Alternatives that have been suggested are ethanol,[12] propylene carbonate/ethanol,[13, 14] propylene carbonate/methanol,[15] ethyl lactate,[16] and ethanol:methyl acetate (75:25).[17]

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ACID HALIDES AND ANHYDRIDES

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Acid halides, sulfonyl chlorides, and anhydrides are widely used in organic chemistry. The safe disposal of a number of these compounds has been investigated.

Compound	Formula	bp or mp	Registry Number
Acetyl chloride[1]	$CH_3C(O)Cl$	bp 52 °C	[75-36-5]
Propionyl chloride[2]	$CH_3CH_2C(O)Cl$	bp 77–79 °C	[79-03-8]
Dimethylcarbamoyl chloride[3]	$(CH_3)_2NC(O)Cl$	bp 167–168 °C	[79-44-7]
Benzoyl chloride[4]	$PhC(O)Cl$	bp 198 °C	[98-88-4]
Thionyl chloride[5]	$SOCl_2$	bp 79 °C	[7719-09-7]
Sulfuryl chloride[6]	SO_2Cl_2	bp 68–70 °C	[7791-25-5]
Methanesulfonyl chloride[7]	CH_3SO_2Cl	bp 60 °C/21 mmHg	[124-63-0]
Benzenesulfonyl chloride[8]	$PhSO_2Cl$	bp 251–252 °C	[98-09-9]
p-Toluenesulfonyl chloride[9]	p-$CH_3C_6H_4SO_2Cl$	mp 67–69 °C	[98-59-9]
Acetic anhydride[10]	$(CH_3C(O))_2O$	bp 138–140 °C	[108-24-7]

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All of these compounds are corrosive, can cause burns, and some may be lachrymators. In general, these compounds react violently with dimethyl sulfoxide (DMSO).[11] A number of other incompatibilities have been noted, for example, acetyl chloride reacts violently with ethanol,[12] propionyl chloride reacts violently with diisopropyl ether,[13] thionyl chloride reacts violently with a variety of reagents including ammonia, *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), and ethanol,[14] sulfuryl chloride is incompatible with lead(IV) oxide, ether, red phosphorus, dinitrogen pentaoxide, and alkalies;[15] benzenesulfonyl chloride reacts violently with methylformamide;[16] and acetic anhydride reacts violently with a variety of compounds including boric acid, chromium trioxide, ethanol, nitric acid, and perchloric acid.[17] This list is not exhaustive and standard reference works should be consulted before proceeding.[18] Benzenesulfonyl chloride may explode on storage.[16] These compounds all react readily, and sometimes violently, with H_2O, alcohols, and amines. Dimethylcarbamoyl chloride is carcinogenic in experimental animals[19] and sulfuryl chloride may be carcinogenic.[20]

Principle of Destruction

Under controlled conditions, these compounds are readily hydrolyzed to the corresponding acids. Highly reactive compounds (e.g., acetyl chloride, propionyl chloride, dimethylcarbamoyl chloride, benzoyl chloride, thionyl chloride, sulfuryl chloride, methanesulfonyl chloride, and acetic anhydride) are simply added to a 2.5 M sodium hydroxide (NaOH) solution at room temperature, while compounds of lesser reactivity (e.g., benzenesulfonyl chloride and *p*-toluenesulfonyl chloride) require prolonged stirring or refluxing with a 2.5 M NaOH solution. For these compounds, destruction was >99.98%. Chlorosulfonic acid is too reactive to be degraded using any of these procedures (see the monograph on Chlorosulfonic Acid).

Destruction Procedures

Destruction Procedure for Highly Reactive Compounds (e.g., Acetyl Chloride, Propionyl Chloride, Dimethylcarbamoyl Chloride, Benzoyl Chloride, Thionyl Chloride, Sulfuryl Chloride, Methanesulfonyl Chloride, and Acetic Anhydride)[21]

Cautiously add 5 mL or 5 g of the compound to 100 mL of a 2.5 M NaOH solution. Stir the reaction at room temperature until it is over (it may be useful to monitor the temperature), and then neutralize, and discard it.

Destruction Procedure for Compounds of Lesser Reactivity (e.g., Benzenesulfonyl Chloride and p-Toluenesulfonyl Chloride)[21]

1. Add 5 mL or 5 g of the compound to 100 mL of a 2.5 M NaOH solution. Cover and stir the reaction at room temperature for 3 h (benzenesulfonyl chloride) or 24 h (*p*-toluenesulfonyl chloride), analyze for completeness of destruction, neutralize the reaction mixture, and discard it.

2. Add 5 mL or 5 g of the compound to 100 mL of a 2.5 M NaOH solution. Reflux the reaction mixture for 1 h, cool, analyze for completeness of destruction, neutralize the reaction mixture, and discard it.

Destruction Procedure for Compounds of Unknown Reactivity[22]

To degrade 0.5 mol of the compound, stir a NaOH solution (2.5 M, 600 mL) in a 1 L flask and add a few milliliters of the compound. If the compound dissolves and heat is generated, add the rest of the compound at such a rate that the reaction remains under control. If the reaction is slow (e.g., with *p*-toluenesulfonyl chloride), heat the mixture to about 90 °C (e.g., with a steam bath), and when the compound has dissolved, add the rest of the compound dropwise. When a clear solution is obtained, allow it to cool. Neutralize the final cooled, reaction mixture, analyze for completeness of destruction, and discard it.

Analytical Procedures[21]

The following procedure has been found useful for the analysis of benzenesulfonyl chloride and *p*-toluenesulfonyl chloride. A 100-μL aliquot of the reaction mixture is neutralized by adding it to 1 mL of a 20 μL/mL solution of acetic acid in methanol. Analyze by reverse phase HPLC using acetonitrile:water (60:40) flowing at 1 mL/min and a UV detector set at 254 nm. The approximate retention times are 6.5 min for benzenesulfonyl chloride and 7.6 min for *p*-toluenesulfonyl chloride. Impurities in the *p*-toluenesulfonyl chloride may interfere with the analysis.

Related Compounds

This procedure should be generally applicable to acid halides, sulfonyl halides, and acid anhydrides. Chlorosulfonic acid is, however, too reactive to be treated by any of these methods. See the monograph on Chlorosulfonic Acid.

References

1. Other names for this compound are ethanoyl chloride, acetic acid chloride, and acetic chloride.
2. Other names for this compound are propanoyl chloride and propionic chloride.
3. Other names for this compound are chloroformic acid dimethylamide, DDC, (dimethylamino)carbonyl chloride, dimethylcarbamic acid chloride, dimethylcarbamic chloride, dimethylcarbamidoyl chloride, *N,N*-dimethylcarbamoyl chloride, dimethylcarbamyl chloride, *N,N*-dimethylcarbamyl chloride, and DMCC.
4. Other names for this compound are benzenecarbonyl chloride, benzoic acid chloride, or α-chlorobenzaldehyde.
5. Other names for this compound are sulfinyl chloride, sulfurous oxychloride, sulfur chloride oxide, sulfurous dichloride, and thionyl dichloride.
6. Other names for this compound are sulfonyl chloride and sulfuric oxychloride.
7. Another name for this compound is mesyl chloride.

8. Other names for this compound are benzene sulfone chloride, benzenesulfonic acid chloride, and benzenosulphochloride.
9. Other names for this compound are 4-methylbenzenesulfonyl chloride, tosyl chloride, and toluenesulfonic acid chloride.
10. Other names for this compound are acetic oxide, acetyl oxide, ethanoic anhydrate, acetyl ether, acetyl anhydride, and acetic acid anhydride.
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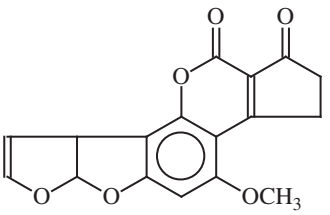
AFLATOXINS

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Aflatoxins are fungal metabolites produced by *Aspergillus parasiticus* and *Aspergillus flavus*. In hot humid areas, peanuts, beans, and corn may be contaminated with aflatoxins. A variety of aflatoxins are known, and all are high-melting (>180 °C) crystalline solids. The most commonly encountered aflatoxins are B_1 [1162-65-8], B_2 [7220-81-7], G_1 [1165-39-5], G_2 [7241-98-7], and M_1 [6795-23-9] (which is the major metabolite of aflatoxin B_1 in milk). Other aflatoxins are known. These aflatoxins are all chemically very similar[1] and the structure of aflatoxin B_1 is shown below



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Aflatoxin B_1

Aflatoxins are carcinogenic in humans and laboratory animals.[2] These compounds are also acutely poisonous by ingestion,[3] are used in the laboratory for cancer research, and are found as analytical standards in laboratories doing surveillance of foodstuffs. Solid aflatoxins may become electrostatically charged and cling to glassware or protective clothing.

Principles of Destruction

Aflatoxins may be degraded using ammonia (NH_3),[4] potassium permanganate in sulfuric acid ($KMnO_4$ in H_2SO_4),[4] potassium permanganate in 2 M sodium hydroxide solution ($KMnO_4$ in NaOH),[5] or 5.25% sodium hypochlorite (NaOCl) solution followed by the addition of acetone.[4] The acetone is required to destroy any 2,3-dichloroaflatoxin B_1 that may have been formed by the action of the NaOCl. Before the addition of the acetone, the NaOCl concentration should be reduced to 1.3% or less so that the haloform reaction does not occur.[6] When $KMnO_4$ is used, the final reaction mixtures should be made strongly basic and filtered to remove manganese compounds.[7] Animal carcasses may be decontaminated by burying them in quicklime (calcium oxide).[4] Ozone may also be used to degrade aflatoxins in solution.[8]

Note: Sodium hypochlorite has been shown to degrade aflatoxins but, given the propensity of sodium hypochlorite to react to form toxic by-products (see the chapter on the use of sodium hypochlorite near the beginning of this book), it may not be advisable to use this procedure. Other procedures described here, such as potassium permanganate, might be more appropriate.

Destruction Procedures

Destruction of Stock Quantities

1. Add sufficient methanol (ca. 1 mL or more if required) to solubilize the aflatoxins and wet the glassware, then add 2 mL of 5.25% NaOCl solution (see below for assay procedure) for each microgram (μg) of aflatoxin. Allow this to stand overnight, then add three volumes of H_2O and a volume of acetone equal to 5% of the total diluted volume. After 30 min, check for completeness of destruction and discard it.
2. Add sufficient H_2O so that the aflatoxins are dissolved and their concentration does not exceed 2 μg/mL. Then, for each 100 mL of this solution, **cautiously** add 10 mL of concentrated H_2SO_4 with stirring (**exothermic reaction!**). Add 16 g of $KMnO_4$ per liter of the resulting solution. The purple color should remain for at least 3 h. If it does not, add more $KMnO_4$. Leave it to react further for 3 h, then decolorize it with sodium metabisulfite, make it strongly basic by adding 10 M KOH solution (**Caution!** Exothermic!), dilute with H_2O, filter, test the filtrate for completeness of destruction, and discard it.

3. Prepare a 0.3 M solution of $KMnO_4$ in 2 M NaOH solution by stirring the mixture for at least 30 min but no more than 2 h. Dissolve 300 µg of aflatoxins in 5 mL of acetonitrile and add 10 mL of $KMnO_4$ in NaOH. Stir for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of water, and filter to remove the manganese salts, check for completeness of destruction, and discard the solid and filtrate appropriately.

Destruction of Aflatoxins in Aqueous Solution

1. For each microgram of aflatoxin, add 2 mL of 5.25% NaOCl solution (see below for assay procedure). Allow it to stand overnight, then add three volumes of H_2O and a volume of acetone equal to 5% of the total diluted volume. After 30 min, check for completeness of destruction and discard it.
2. For each 100 mL of solution, **cautiously** add 10 mL of concentrated H_2SO_4 with stirring (**exothermic reaction!**). Add 16 g of $KMnO_4$ per liter of the resulting solution. The purple color should remain for at least 3 h. If it does not, add more $KMnO_4$. Leave it to react further for 3 h, then decolorize it with sodium metabisulfite, make it strongly basic by adding 10 M KOH solution (**Caution!** Exothermic!), dilute with H_2O, filter, test the filtrate for completeness of destruction, and discard it.
3. Dilute with H_2O, if necessary, so that the concentration of aflatoxins does not exceed 200 µg/mL. Add sufficient NaOH, with stirring, to make the concentration 2 M, then add sufficient solid $KMnO_4$ to make the concentration 0.3M. Stir for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of water, filter to remove the manganese salts, check for completeness of destruction, and discard the solid and filtrate appropriately.

Destruction of Aflatoxins in Volatile Organic Solvents

1. Evaporate to dryness under reduced pressure using a rotary evaporator (add an equal volume of dichloromethane to DMSO solutions before evaporation), then solubilize the residual aflatoxins in a little methanol (ca. 1 mL). For each microgram of aflatoxin, add 2 mL of 5.25% NaOCl solution (see below for assay procedure). Allow it to stand overnight, then add three volumes of H_2O and a volume of acetone equal to 5% of the total diluted volume. After 30 min, check for completeness of destruction and discard it.

2. Evaporate to dryness under reduced pressure using a rotary evaporator (add an equal volume of dichloromethane to DMSO solutions before evaporation), then dissolve the residual aflatoxins in H_2O (10 mL for each 20 µg of aflatoxins; more if required). For each 100 mL of this solution, **cautiously** add 10 mL of concentrated H_2SO_4 with stirring (**exothermic reaction!**). Add 16 g of $KMnO_4$ per liter of the resulting solution. The purple color should remain for at least 3 h. If it does not, add more $KMnO_4$. Leave it to react further for 3 h, then decolorize it with sodium metabisulfite, make it strongly basic by adding 10 M KOH solution (**Caution!** Exothermic), dilute with H_2O, filter, test the filtrate for completeness of destruction, and discard it.
3. Prepare a 0.3 M solution of $KMnO_4$ in 2 M NaOH solution by stirring the mixture for at least 30 min but no more than 2 h. Remove the organic solvent under reduced pressure using a rotary evaporator (add an equal volume of dichloromethane to DMSO solutions before evaporation). Dissolve 300 µg of aflatoxins in 5 mL of acetonitrile and add 10 mL of $KMnO_4$ in NaOH. Stir for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of H_2O, filter to remove the manganese salts, check for completeness of destruction, and discard the solid and filtrate appropriately.

Destruction of Aflatoxins in Oil

Add 2 mL of a 5.25% NaOCl solution (see below for assay procedure) for each microgram of aflatoxin, shake the mixture on a mechanical shaker for at least 2 h, add three volumes of H_2O for each volume of NaOCl used, then add a volume of acetone equal to 5% of the total diluted volume. After 30 min, check for completeness of destruction and discard it.

Decontamination of Equipment and Thin-Layer Chromatography Plates

First, rinse equipment with a little methanol to solubilize the aflatoxins. Immerse equipment, thin-layer chromatography (TLC) plates, protective clothing, and absorbent paper in a 1:3 mixture of 5.25% NaOCl solution (see below for assay procedure) and H_2O for at least 2 h, then add an amount of acetone equal to 5% of the total volume, allow the mixture to react for at least 30 min, and discard it.

Treatment of Spills

1. First remove as much of the spill as possible by high-efficiency particulate air (HEPA) vacuuming (not sweeping), then rinse the area with a little methanol to solubilize the aflatoxins. Take up the rinse with absorbent paper. Immerse the absorbent paper in a 1:3 mixture of a 5.25% NaOCl solution (see below for assay procedure) and H_2O for at least 2 h, then add an amount of acetone equal to 5% of

the total volume, allow the mixture to react for at least 30 min, and discard it. Wash the surface from which the spill has been removed with a 5.25% NaOCl solution and leave it for 10 min before adding a 5% aqueous solution of acetone.

2. Prepare a 0.3 M solution of $KMnO_4$ in 2 M NaOH solution by stirring the mixture for at least 30 min but no more than 2 h. Collect spills of liquid with a dry tissue and spills of solid with a tissue wetted with dichloromethane. Immerse all tissues in the $KMnO_4$ in NaOH solution. Allow it to react for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of H_2O, filter, check for completeness of destruction, and discard the solid and filtrate appropriately. Cover the spill area with an excess of the $KMnO_4$ in NaOH solution and allow it to react for 3 h. Collect the solution on a tissue and immerse the tissue in 2 M sodium metabisulfite solution. If the pH of this solution is acidic, make it alkaline with NaOH. Rinse the spill area with a 2 M solution of sodium metabisulfite. Check the surface for completeness of decontamination using a wipe moistened with methanol and analyze the wipe for the presence of aflatoxins.

Destruction of Aflatoxins in Animal Litter

Spread the litter on a metal tray to a maximum depth of about 5 cm, then sprinkle it with a 5% NH_3 solution (30–40 mL per 25 g of litter). Autoclave the tray for 20 min at 128–130 °C, then discard the litter. **Do not** pre-evacuate the autoclave as this would remove the NH_3.

Destruction of Aflatoxins in Animal Carcasses

Bury carcasses in quicklime and cover to a depth of about 1 cm.

Destruction of Aflatoxins in Aqueous Solution Using Ozone

Caution! Ozone is an irritant! These reactions should be carried out in a properly functioning fume hood. Pass ozone (20% in oxygen) from an ozone generator through a 32 μM solution of aflatoxins in water, check for completeness of destruction and discard the solution.[8] The authors report complete destruction in 15 s. A bioassay using *Hydra attenuata* showed no residual toxicity.

Analytical Procedures

1. Extract 200 mL of decontaminated waste solution three times with 50 mL portions of chloroform and combine the extracts.[4] Concentrate the extracts to about 3 mL using a rotary evaporator and add this solution to a graduated tube. Wash the flask twice with 2-mL portions of chloroform and add these washes to the tube.

Concentrate the contents of the tube at about 60 °C to 0.5 mL under a stream of nitrogen. Spot a TLC plate with 10 µL of this solution and with 5 µL of a 0.2 mg/L standard solution of aflatoxins and develop with a mixture of chloroform:acetone (9:1) in subdued light. Determine the presence or absence of aflatoxins by visualizing under ultraviolet (UV) light (365 nm). (The TLC plates used were Kieselgel 60 Merck.) More cleanup of the sample may be required, prior to TLC, if the sample is highly colored or if the aflatoxins were initially dissolved in oil. The cleaned sample may also be analyzed by HPLC as described below.

2. For reaction mixtures obtained using the $KMnO_4$ in NaOH procedure, acidify an aliquot to pH 2–3 using concentrated hydrochloric acid (HCl). Extract this mixture three times with an equal volume of dichloromethane, pool the extracts, and dry them over anhydrous sodium sulfate.[5] Remove the sodium sulfate by filtration, evaporate to dryness, and take up the residue in 0.5 mL of water:methanol:acetonitrile (2:1:1). Analyze by reverse phase HPLC using water:methanol:acetonitrile (2:1:1) flowing at 1 mL/min with spectrofluorimetric detection (excitation 360 nm, emission 440 nm).
3. Other analytical techniques have been reviewed[9, 10] and techniques using reverse-phase HPLC with a 10-µm Spherisorb ODS column, water:acetonitrile:-methanol (15:3:2), fluorescence detection with excitation 365 nm, emission about 450 nm,[11] a C_{18} µBondapak column, methanol:water (40:60), fluorescence detection with excitation 360 nm and a 417 nm cut-off emission filter,[12] and a Zorbax SB-C18 column, methanol:water 35:65, fluorescence detection with excitation 360 nm and emission 440 nm[13] have been described. Methods using UPLC-MS,[14, 15] and postcolumn derivatization detection[16] have been reported.

Mutagenicity Assays

Aflatoxins B_1 and G_1 have been shown to be mutagenic in *Salmonella typhimurium* and other species,[17] but specific studies of possible mutagenic products from the degradation procedures involving NH_3, $KMnO_4$ in H_2SO_4, and 5.25% NaOCl solution followed by the addition of acetone have not been carried out. The residues from the degradation reactions involving $KMnO_4$ in NaOH were tested for mutagenicity using tester strains TA97, TA98, TA100, and TA102 of *S. typhimurium*.[5] No mutagenic activity was found.

Related Compounds

The abovementioned techniques were investigated for aflatoxins B_1, B_2, G_1, and G2, but they should also be applicable to other aflatoxins. Methods for decontaminating aflatoxins on peanuts have been reviewed.[18] Some of these methods may be of use in the laboratory. The method using ozone[8] has also been shown to degrade cyclopiazonic acid, ochratoxin A, patulin, secalonic acid, and zearalenone. However, although fumonisin B_1 was completely degraded, the bioassay using *Hydra attenuata* showed residual toxicity.

Assay of Sodium Hypochlorite Solution

Sodium hypochlorite solutions tend to deteriorate with time, so they should be periodically checked for the amount of active chlorine they contain. Pipette 10 mL of the NaOCl solution into a 100 mL volumetric flask and fill it to the mark with distilled H_2O. Pipette 10 mL of this solution into a conical flask containing 50 mL of distilled H_2O, 1 g of potassium iodide (KI), and 12.5 mL of 2 M acetic acid. Titrate this solution against a 0.1 N sodium thiosulfate solution using starch as an indicator. Each 1 mL of the sodium thiosulfate solution corresponds to 3.545 mg of active chlorine. Commercially available NaOCl solution (Clorox bleach) contains 5.25% NaOCl and should contain 45–50 g of active chlorine per liter.

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ALKALI AND ALKALINE EARTH METALS

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

The alkali metals sodium (Na) [7440-23-5],[1, 2] potassium (K) [7440-09-7],[3, 4] and lithium (Li) [7439-93-2][5, 6] react violently with H_2O or even moist air to generate hydrogen, which can then be ignited by the heat of the reaction. These metals are corrosive to the skin and incompatible with many organic and inorganic compounds, including halocarbons. Potassium may oxidize on storage and oxidized metal may explode violently when handled or cut.[4, 7] These metals are used in organic synthesis and as drying agents, although they should not be used to dry halogenated solvents.[8] Alkali metals require special fire-extinguishing procedures. The alkaline earth metals magnesium (Mg) [7439-95-4],[9, 10] calcium (Ca) [7440-70-2],[11, 12] strontium (Sr) [7440-24-6],[13, 14] and barium (Ba) [7440-39-3][15, 16] are less reactive to water, but they are incompatible with many organic and inorganic compounds. Magnesium and barium have been reported to be incompatible with halocarbons.

Principles of Destruction

The alkali metals are allowed to react with an alcohol in a slow and controlled fashion to generate the metal alkoxide and hydrogen. The choice of alcohol is critical, see below.

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The metal alkoxide is subsequently hydrolyzed with H_2O to give the metal hydroxide and alcohol. A method involving dropping lumps of K into a hole in the ground partially filled with water[17] would probably not be acceptable in today's regulatory climate. Barium, calcium, and strontium are allowed to react with H_2O to generate the metal hydroxide and hydrogen. It has been reported[11] that when calcium reacts with H_2O, the heat of the reaction may ignite the hydrogen that is evolved. However, we have not experienced any problems with this procedure. Magnesium is allowed to react with dilute hydrochloric acid (HCl) to generate magnesium chloride and hydrogen. In all cases, the hydrogen is vented into the fume hood.

A procedure for recycling scrap pieces of sodium has been published.[18]

Destruction Procedures

Caution! These procedures present a high fire hazard and should be conducted in a properly functioning chemical fume hood away from flammable solvents. The presence of a nonflammable board or cloth for smothering the reaction, as well as an appropriate fire extinguisher, may be advisable. If possible, do the reaction in batches to minimize the risk.

Sodium and Lithium

Add 1 g of Na or Li to 100 mL of cold ethanol at such a rate that the reaction does not become violent.[19] Stir the reaction mixture. If the reaction mixture becomes viscous and the rate of reaction slows, add more ethanol. When all the metal has been added, stir the reaction mixture until all reaction ceases, then examine carefully for the presence of unreacted metal. If none is found, dilute the mixture with H_2O, neutralize, and discard it.

Potassium

Potassium is the most treacherous of the alkali metals, and fires during its destruction are not infrequent. Precautions for its safe handling have been described.[20–22] For an entertaining description of what can happen, see the chapter on potassium in Primo Levi's book "The Periodic Table."[23]

Add the K to *tert*-butyl alcohol[20] at a rate so that the reaction does not become violent. If the reaction mixture becomes viscous and the rate of reaction slows, add more *tert*-butyl alcohol. When all the K has been added, stir the reaction mixture until all reaction ceases, then examine *carefully* for the presence of unreacted metal. If none is found, dilute the mixture with H_2O, neutralize, and discard. *tert*-Amyl alcohol may also be used.[21] Whichever alcohol is used, it is important to use an anhydrous grade. If necessary, the alcohol should be dried before use. Powdered 3 Å molecular sieve has been recommended.[24]

Magnesium

Add 1 g of Mg to 100 mL of 1 M HCl and stir the mixture.[19] When the reaction has ceased, neutralize the reaction mixture, and discard it.

Barium, Calcium, and Strontium

Add 1 g of Ba, Ca, or Sr metal to 100 mL of H_2O in portions and stir the mixture.[19] When the reaction has ceased, neutralize the reaction mixture, and discard it.

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ALKALI METAL ALKOXIDES

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

The alkali metal alkoxides sodium methoxide (sodium methylate, CH_3ONa) [124-41-4], sodium ethoxide (sodium ethylate, CH_3CH_2ONa) [141-52-6], and potassium *tert*-butoxide [$(CH_3)_3COK$] [865-47-4] are corrosive. Sodium methoxide[1] and sodium ethoxide[2] may ignite in moist air. Sodium methoxide reacts violently with chloroform,[3] and $(CH_3)_3COK$ ignites on contact with acids or reactive solvents.[4] These compounds are used in organic synthesis.

Principle of Destruction

The alkali metal alkoxides are hydrolyzed with H_2O to sodium or potassium hydroxide (NaOH or KOH) and the corresponding alcohol.

Destruction Procedure

Add 5 g of the alkoxide to 100 mL of H_2O and stir the mixture. When all the alkoxide has dissolved and the reaction appears to be over, discard the mixture.[5]

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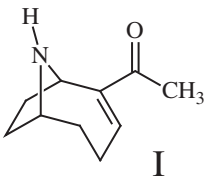
ANATOXIN-A

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Anatoxin-a[1] [**I**, 64285-06-9, racemic mixture] is a naturally occurring acute neurotoxin produced by various species of freshwater cyanobacteria within the genera of *Anabaena* (especially *flos-aquae*), *Aphanizomenon*, *Microcystis*, *Planktothrix*, and *Oscillatoria*.[2–4] It is soluble in water and rapidly degrades in sunlight and alkaline solution.[5, 6]



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The fumarate is a light brown hygroscopic solid which melts with decomposition at 124–126 °C.[7]

Anatoxin-a is toxic by ingestion in a variety of animals,[2, 8] a poison by the intraperitoneal route,[9] and a teratogen.[9] It is irritating to the eyes, respiratory system, and skin. Adverse reproductive effects have been observed in hamsters.[8]

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Principles of Destruction

Anatoxin-a may be oxidized using ozone[10, 11] or potassium permanganate.[11] Destruction is >95%. The products of these reactions are not known.

Destruction Procedures

Destruction Using Ozone

Caution! Ozone is an irritant! These reactions should be carried out in a properly functioning fume hood.

Add ozone to a 20 µg/L solution of anatoxin-a in water so as to achieve an ozone concentration of 2 mg/L, shake vigorously, after 5 min test for completeness of destruction, and discard it.[10] In another report,[11] a 166 µg/L solution of anatoxin-a in water was degraded by adding ozone to a final concentration of 1 mg/L. These experiments were carried out with natural lake waters having pH 7–8 and dissolved organic carbon of 3.6–15.5 mg/L. Other organic species may influence the efficiency of destruction.

Using Potassium Permanganate

Add a 10 mM solution of potassium permanganate in water to a 166 µg/L solution of anatoxin-a in water to a final potassium permanganate concentration of 0.5 mg/L, check for completeness of destruction, and discard it.[11] These experiments were carried out with natural lake water having pH 8 and dissolved organic carbon of 3.6 mg/L. Other organic species may influence the efficiency of destruction.

Analytical Procedures

Anatoxin-a may be determined by HPLC using a 250 mm × 4.6 mm 5 µm Phenomenex Gemini C18 column and a mobile phase of MeCN:water:trifluoroacetic acid (3.5:96.5:0.05) flowing at 0.9 mL/min.[12] The retention time is approximately 9 min and with UV detection at 225 nm the limit of detection is 33 µg/L. It might be possible to increase this limit of detection, for example, by using high volume injections. A more sensitive method using solid-phase extraction and derivatization has also been reported. Condition a 3 mL Supelco WCX weak cation-exchange SPE cartridge with 6 mL MeOH and 6 mL water.[13] Add 10 mL of a filtered water sample, adjusted to pH 7, to the cartridge, wash with 3 mL MeOH:water (50:50), and dry with air. Elute with 10 mL MeOH:trifluoroacetic acid (99.8:0.2). Evaporate the eluate at 50 °C under nitrogen and reconstitute with 100 µL 100 mM sodium borate solution. In an amber vial react with 50 µL 1 mg/mL 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) in MeCN in the dark for 10 min. Add 50 µL 1 M HCl and inject a 20 µL aliquot onto a 250 mm × 3.2 mm 5 µm Phenomenex Ultremex or Prodigy C18 column at 35 °C. Elute with MeCN:water (45:55) flowing at 0.5 mL/min and monitor with a fluorescence detector, excitation 470 nm, emission 530 nm. The retention time is approximately 15 min, and the limit of detection is 10 ng/L. A similar procedure using methanol instead of acetonitrile in the mobile phase has been described by Azevedo et al.[14] A limit of detection of 0.65 ng/L has been reported using an optimized HPLC-MS procedure.[15]

Related Compounds

These procedures are specific for anatoxin-a.

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AROMATIC AMINES

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Aromatic amines constitute a group of widely used synthetic organic chemicals. Many have been shown to be carcinogens in experimental animals, and a number of them are thought to be human carcinogens. 4-Aminobiphenyl (4-ABP) [92-67-1],[1, 2] benzidine (Bz) [92-87-5],[3–5] and 2-naphthylamine (2-NAP) [91-59-8][6, 7] are human and animal carcinogens and 3,3′-dichlorobenzidine (DClB) [91-94-1],[8–10] 3,3′-dimethoxybenzidine (DMoB) [119-90-4],[11, 12] di-(4-amino-3-chlorophenyl) methane (MOCA) [101-14-4],[13] 3,3′-dimethylbenzidine (DMB) [119-93-7],[14] 2,4-diaminotoluene (TOL) [95-80-7],[15] and 2-aminoanthracene (2-AA) [613-13-8][16, 17] cause cancer in laboratory animals. Diaminobenzidine (DAB) [91-95-2] may cause cancer in experimental animals.[18] Benzidine may cause damage to the blood.[19]

In a collaborative study organized by the International Agency for Research on Cancer (IARC) on the laboratory destruction of aromatic amines,[20] the following aromatic amines were considered: 4-ABP,[21] benzidine (Bz; **I**, R = H),[22] DClB (**I**, R = Cl),[23] DMoB (**I**, R = OCH_3),[24] DMB (**I**, R = CH_3),[25] MOCA,[26] 1-naphthylamine (1-NAP) [134-32-7],[27] 2-NAP,[28] and TOL.[29] Procedures for the destruction of DAB[30] (**I**, R = NH_2)[31, 32] and 2-AA[33] (**II**)[34] have also been published.

Destruction of Hazardous Chemicals in the Laboratory, Fourth Edition. George Lunn and Eric B. Sansone.
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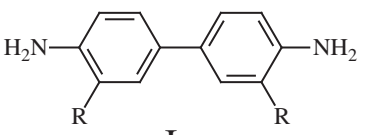
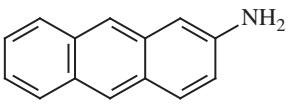


I



II

All of these compounds are crystalline solids and are generally very sparingly soluble in cold H_2O, more soluble in hot H_2O, and very soluble in acid and organic solvents. Some of these compounds are used in the chemical industry. Aromatic amines are also used in chemical laboratories and in biomedical research, for example, as stains and analytical reagents.

Note: Unless otherwise stated all of these procedures can be used for all of the amines mentioned above except for 2-aminoanthracene.

Principles of Destruction

Aromatic amines may be oxidized with potassium permanganate in sulfuric acid ($KMnO_4$ in H_2SO_4).[20, 32] The products of this reaction have not been identified. Aromatic amines may also be removed from solution using horseradish peroxidase in the presence of hydrogen peroxide (H_2O_2).[20, 32, 35] The enzyme catalyzes the oxidation of the aromatic amine to a radical. These radicals diffuse into solution and polymerize. The polymers are insoluble and fall out of solution. Although the resulting solution is nonmutagenic, the precipitated polymer is mutagenic so this method was only recommended for the treatment of large quantities of aqueous solution containing small amounts of aromatic amines.[20] In all cases, destruction was >99%. We have also found that the above procedures may also be applied to DAB.[32] Additionally, we found that residual amounts of H_2O_2 in the horseradish peroxidase procedure produced a mutagenic response and that the mutagenicity could be removed by adding ascorbic acid solution (to reduce the H_2O_2) to the final reaction mixtures.[32] A related horseradish peroxidase catalyzed reaction can be used to degrade pentachlorophenol.[36] A paper by Anotai et al.[37] showed that 2,6-dimethylaniline and *o*-toluidine (2-methylaniline) can be degraded using the Fenton reaction. 2,6-Dimethylaniline[38] and aniline[39] are suspected carcinogens and *o*-toluidine[40] is a confirmed carcinogen. The products of this reaction have not been identified. Various procedures involving diazotization followed by decomposition of the diazo compound have been investigated, but the results seem to depend on the nature of the aromatic amine.[20] These procedures are not discussed here.

Destruction Procedures

Destruction of Aromatic Amines in Bulk and in Organic Solvents[20, 32]

Evaporate solutions of aromatic amines in organic solvents to dryness using a rotary evaporator. Dissolve the aromatic amines as follows: for each 9 mg of DAB tetrahydrochloride dihydrate, dissolve in 10 mL of H_2O, for each 9 mg of Bz, DAB,

DClB, DMB, DMoB, 1-NAP, 2-NAP, and TOL, dissolve in 10 mL of 0.1 M HCl; for each 2.5 mg of MOCA, dissolve in 10 mL of 1 M H_2SO_4; for each 2 mg of 4-ABP, dissolve in 10 mL of glacial acetic acid; for each 2 mg of mixtures of the above amines, add 10 mL of glacial acetic acid. Stir these solutions until the aromatic amines have completely dissolved, then for each 10 mL of the solution so formed, add 5 mL of 0.2 M $KMnO_4$ solution and 5 mL of 2 M H_2SO_4. Allow the mixture to stand for at least 10 h, and then analyze for completeness of destruction. Decolorize the mixture by the addition of sodium metabisulfite, make it strongly basic by the addition of 10 M KOH solution (**Caution!** Exothermic), dilute with H_2O, filter to remove manganese compounds,[41] check the filtrate for completeness of destruction, neutralize, and discard it.

Destruction of Aromatic Amines in Aqueous Solution[20, 32]

Dilute with H_2O, if necessary, so that the concentration of MOCA does not exceed 0.25 mg/mL, the concentration of 4-ABP does not exceed 0.2 mg/mL, and the concentration of the other amines does not exceed 0.9 mg/mL. For each 10 mL of solution, add 5 mL of 0.2 M $KMnO_4$ solution and 5 mL of 2 M H_2SO_4 solution. Allow the mixture to stand for at least 10 h, then analyze for completeness of destruction. Decolorize the mixture by the addition of sodium metabisulfite, make it strongly basic by the addition of 10 M KOH solution (**Caution!** Exothermic), dilute with H_2O, filter to remove manganese compounds,[41] check the filtrate for completeness of destruction, neutralize, and discard it.

Destruction of Aromatic Amines in Oil[20]

Extract the oil solution with 0.1 M HCl until all the amines are removed (at least 2 mL of HCl will be required for each micromole of amine). For each 10 mL of HCl solution, add 5 mL of 0.2 M $KMnO_4$ solution and 5 mL of 2 M H_2SO_4 solution. Allow the mixture to stand for at least 10 h, then analyze for completeness of destruction. Decolorize the mixture by the addition of sodium metabisulfite, make it strongly basic by the addition of 10 M KOH solution (**Caution!** Exothermic), dilute with H_2O, filter to remove manganese compounds,[41] check the filtrate for completeness of destruction, neutralize, and discard it.

Destruction of 2-Aminoanthracene[34]

Evaporate solutions in organic solvents to dryness using a rotary evaporator. Prepare a 0.3 M solution of $KMnO_4$ in 3 M H_2SO_4 by stirring $KMnO_4$ in 3 M H_2SO_4 (47.4 g $KMnO_4$ for each liter of acid) for at least 15 min but no longer than 1 h. Dissolve the 2-aminoanthracene in glacial acetic acid so that the concentration does not exceed 10 mg/mL. Stir until the 2-aminoanthracene has completely dissolved, then for each 1 mL of the solution so formed, add 40 mL of 0.3 M $KMnO_4$ in 3 M H_2SO_4 and stir the mixture for at least 18 h. Decolorize the mixture by the addition of sodium metabisulfite, make it strongly basic by the addition of 10 M KOH solution (**Caution!**

Exothermic!), dilute with H_2O, filter to remove manganese compounds,[41] check the filtrate for completeness of destruction, neutralize, and discard it.

Decontamination of Spills[20]

Remove as much of the spill as possible by the use of absorbents and HEPA vacuuming, then wet the surface with glacial acetic acid until all the amines are dissolved. Add an excess of a mixture of equal volumes of 0.2 M $KMnO_4$ solution and 2 M H_2SO_4 to the spill area. Allow the mixture to stand for at least 10 h, decolorize with sodium metabisulfite (while ventilating the area), and mop up the liquid with paper towels. Squeeze the solution out of the towels, basify, dilute with H_2O, filter to remove manganese compounds,[41] and test the filtrate for completeness of destruction. Test for completeness of decontamination by wiping the surface with a wipe moistened with an appropriate solvent.

Decontamination of Glassware[20]

Immerse the glassware in a mixture of equal volumes of 0.2 M $KMnO_4$ and 2 M H_2SO_4. Allow the glassware to stand in the bath for at least 10 h, then decolorize the mixture by the addition of sodium metabisulfite, make it strongly basic by the addition of 10 M KOH solution (**Caution!** Exothermic!), dilute with H_2O, filter to remove manganese compounds,[41] check the filtrate for completeness of destruction, neutralize, and discard it.

Decontamination of Large Quantities of Solutions Containing Aromatic Amines[20, 32]

Note: This method is recommended for all the amines listed above except 4-ABP and 2-NAP where destruction was found to be incomplete and 2-aminoanthracene (not tested).

Adjust the pH of aqueous solutions to 5–7 using acid or base as appropriate and dilute so that the concentration of aromatic amines does not exceed 100 mg/L. Dilute solutions in methanol, ethanol, DMSO, or DMF with sodium acetate solution (1 g/L) so that the concentration of organic solvent does not exceed 20% and the concentration of aromatic amines does not exceed 100 mg/L. For each liter of solution, add 3 mL of a 3% solution of H_2O_2 and 300 U of horseradish peroxidase. Allow the mixture to stand for 3 h, then remove the precipitate by filtration or centrifugation. For each liter of filtrate, add 100 mL of a 5% (w/v) ascorbic acid solution. Check the solution for completeness of degradation and discard. The residue is mutagenic and should be treated as hazardous. It has been reported that further oxidation of this residue by $KMnO_4$ in H_2SO_4 produces nonmutagenic residues.[20] Filter the reaction mixture through a porous glass filter and immerse this filter in a 1:1 mixture of 0.2 M aqueous $KMnO_4$ solution and 2 M H_2SO_4 solution.[32, 42]

The horseradish peroxidase used was Donor: hydrogen peroxide oxidoreductase; EC 1.11.1.7 (Type II) having a specific activity of 150–200 purpurogallin units per milligram obtained from Sigma. An appropriate amount was dissolved in sodium acetate

solution (1 g/L), then an aliquot of this solution was used to obtain the requisite number of units.

Destruction of Aromatic Amines Using the Fenton Reaction[37]

Note: This method is recommended for aniline, 2,6-dimethylaniline, and *o*-toluidine.

Adjust a 1 mM solution of the aromatic amine to pH 3 with sulfuric acid or sodium hydroxide, add ferrous sulfate heptahydrate to a final concentration of 1 mM, and add hydrogen peroxide to a final concentration of 20 mM. Allow to react at room temperature for at least 30 (*o*-toluidine) or 60 (aniline, 2,6-dimethylaniline) min, test for completeness of destruction, and discard it.

Analytical Procedures

There are many publications on the analysis of aromatic amines. The following HPLC analysis was recommended by the IARC.[20] A 250 mm × 4.6 mm i.d. reverse phase column was used and the mobile phase was acetonitrile:methanol:buffer (10:30:20) flowing at 1.5 mL/min. The buffer was 1.5 mM in potassium phosphate, dibasic (K_2HPO_4), and 1.5 mM in potassium phosphate, monobasic (KH_2PO_4). If a variable wavelength UV detector is available, the following wavelengths can be used: Bz = 285 nm, DMoB = 305 nm, DMB = 285 nm, DClB = 285 nm, 1-NAP = 240 nm, 2-NAP = 235 nm, 4-ABP = 275 nm, MOCA = 245 nm, and TOL = 235 nm. If only a fixed wavelength detector is available, use 280 nm for the first four and 254 nm for the rest. DAB can be determined using the above buffer:methanol (75:25) flowing at 1 mL/min with the UV detector set at 300 nm.[32] 2-Aminoanthracene was determined using methanol:water (70:30) flowing at 1 mL/min with the UV detector set at 254 nm.[34] Greater sensitivity can be obtained by extracting the basified reaction mixtures with cyclohexane, drying these extracts over anhydrous sodium sulfate, evaporating to dryness, and taking up the residue in a little methanol.

Mutagenicity Assays[20, 32]

Reaction mixtures obtained when the aromatic amines (except for DAB) were degraded with $KMnO_4$ in H_2SO_4 were tested for mutagenicity using *S. typhimurium* strains TA97, TA98, and TA100. No mutagenic activity was seen. Using the same strains, the supernatants from the horseradish peroxidase–H_2O_2 reactions were tested. Again, no mutagenic activity was seen but the solid residues from Bz, DClB, DMoB, and 2-NAP were mutagenic. Reaction mixtures obtained from the degradation of DAB and 2-aminoanthracene were tested using strains TA98, TA100, TA1530, and TA1535 and no mutagenic activity was found.

Related Compounds

The procedures described above should be generally applicable to other aromatic amines, but thorough validation is essential in each case as some variability may be observed, particularly for the horseradish peroxidase method.[20]

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22. Other names for this compound are (1,1′-biphenyl)-4,4′-diamine, 4,4′-biphenyldiamine, 4,4′-diaminobiphenyl, 4,4′-diaminodiphenyl, *p,p*′-dianiline, *p,p*′-bianiline, 4,4′-biphenylenediamine, C.I. 37225, C.I. azoic dye component 112, *p,p*′-dianiline, 4,4′-diphenylenediamine, Fast Corinth Base B, and NCI-C03361.
23. Other names for this compound are 4,4′-diamino-3,3′-dichlorobiphenyl, 3,3′-dichloro-4,4′-biphenyldiamine, 3,3′-dichlorobiphenyl-4,4′-diamine, DCB, 3,3′-dichloro-4,4′-diaminobiphenyl, C.I. 23060, Curithane C126, and 3,3′-dichlorobenzidene.
24. Other names for this compound are 3,3′-dimethoxy(1,1′-biphenyl)-4,4′-diamine, 3,3′-dimethoxy-4,4′-diaminobiphenyl, *o*-dianisidine, *o,o*′-dianisidine, Acetamine Diazo Black RD, Amacel Developed Navy SD, Azoene Fast Blue Base, Azofix Blue B Salt, Azogne Fast Blue B, Blue BN Balse, Brentamine Fast Blue Base, Cellitazol B, C.I. 24110, C.I. Azoic Diazo Component 48, Cibacete Diazo Navy Blue 2B, C.I. Disperse Black 6, Diacelliton Fast Grey G, Diacel Navy DC, Diato Blue Base B, Fast Blue B Base, Hiltonil Fast Blue B Base, Hiltosal Fast Blue B Salt, Hindasol Blue B Salt, Kako Blue B Salt, Kayaku Blue B Base, Lake Blue B Base, Meisei Teryl Diazo Blue HR, Mitsui Blue B Base, Napthanil Blue B Base, Neutrosel Navy BN, Sanyo Fast Blue Salt B, Setacyl Diazo Navy R, and Spectrolene Blue B.
25. Other names for this compound are 3,3′-dimethyl(1,1′-biphenyl)-4,4′-diamine, 3,3′-tolidine, bianisidine, 4,4′-bi-*o*-toluidine, 4,4′-diamino-3,3′-dimethylbiphenyl, 4,4′-diamino-3,3′-dimethyldiphenyl, 3,3′-dimethyl-4,4′-biphenyldiamine, 3,3′-dimethyl-4,4′-diphenyldiamine, 3,3′-dimethylbiphenyl-4,4′-diamine, 3,3′-dimethyldiphenyl-4,4′-diamine, *o*-tolidine, *o,o*′-tolidine, C.I. 37230, C.I. Azoic Diazo Component 113, diaminoditolyl, 4,4′-di-*o*-toluidine, Fast Dark Blue Base R, and *o*-tolidin.
26. Other names for this compound are 4,4′-methylenebis(2-chlorobenzenamine), 4,4′-diamino-3,3′-dichlorodiphenylmethane, 4,4′-methylene(bis)chloroaniline, methylene-4,4′-bis-(*o*-chloroaniline), *p,p*′-methylenebis(α-chloroaniline), 4,4′-methylenebis(*o*-chloroaniline), *p,p*′-methylenebis(*o*-chloroaniline), 4,4′-methylenebis(2-chloroaniline), DACPM, Bis amine, Curalin M, Curene 442, Cyanaset, 3,3′-dichloro-4,4′-diaminodiphenylmethane, and MBOCA.

27. Other names for this compound are 1-naphthalenamine, α-naphthylamine, 1-aminonaphthalene, naphthalidine, and C.I. Azoic Dye Component 114.
28. Other names for this compound are 2-naphthalenamine, β-naphthylamine, 2-aminonaphthalene, 6-naphthylamine, C.I. 37270, Fast Scarlet Base B, NA, 2-naphthalamine, 2-naphthalenamine, and USAF CB-22.
29. Other names for this compound are toluene-2,4-diamine, *m*-tolylenediamine, 3-amino-*p*-toluidine, 5-amino-*o*-toluidine, 1,3-diamino-4-methylbenzene, 2,4-diamino-1-methylbenzene, 4-methyl-1,3-benzenediamine, 4-methyl-*m*-phenylenediamine, 2,4-tolamine, *m*-toluenediamine, 2,4-toluenediamine, *m*-toluylenediamine, 2,4-toluylenediamine, 2,4-tolylenediamine, tolylene-2,4-diamine, Azogen Developer H, Benzofur MT, C.I. 76035, C.I. Oxidation Base, Developer H, Eucanine GB, Fouramine, Fourrine M, MTD, Nako TMT, NCI-C02302, Pelagol Grey J, Pontamine Developer TN, Renal MD, TDA, Zoba GKE, and Zogen Developer H.
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ARSENIC

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Arsenic (As) [7440-38-2] is a human carcinogen.[1] It is also a teratogen and has a number of other adverse biological effects.[2]

Principles of Decontamination

Arsenic(III) may be oxidized to arsenic(V) with Fenton's reagent (hydrogen peroxide in the presence of Fe^{2+} catalyst), and then total inorganic arsenic (up to 2.5 mg/L) may be removed from the solution by ferric (oxy) hydroxide co-precipitation and passing the solution through a column of iron filings. Less than 10 ppb of arsenic remains.[3] Alternatively, arsenic can be removed from water containing up to 750 µg/L arsenic using calcium hypochlorite and ferric sulfate followed by filtration through sand. In almost all cases, <50 ppb arsenic remains.[4] Another method is to filter water containing up to 440 µg/L arsenic through a mixture of sand and iron filings. Again, <50 ppb arsenic remains.[5] Arsenic can be removed from these sand filters by using ethylenediaminetetraacetic acid (EDTA) as a chelating agent.[6] The presence of dissolved oxygen is necessary for this process and a pH of 6 appears to be optimum.[7] The effect of pH and various anions on the process has been investigated.[8, 9] Although the

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filters and precipitates must be disposed of as arsenic-contaminated waste, the total quantity of waste may be reduced using these procedures. As(V) can be removed from the solution using ferrous sulfate and calcium carbonate.[10] The arsenic is removed with the sediment and removal efficiency is 99.64%. The use of titanium dioxide for the removal of arsenic has been reviewed.[11] UV photocatalytic oxidation-coagulation using titanium sulfate can be used to remove arsenic from solution.[12] Diphenylarsinic acid can be photolyzed to a mixture of As(III) and As(V) oxides.[13]

Decontamination Procedures

A. *Using Fenton's reagent*.[3] Add 100 mg ferrous ammonium sulfate and 100 µL 30% hydrogen peroxide to 1 L of water containing 2.5 mg/L of As(III), stir occasionally for 10 min, pass through a 250 mm × 50 mm i.d. column filled with 150 g iron filings at flow rates of up to 150 mL/min, pass through a bed of sand, test for completeness of decontamination, and discard it. Residual hydrogen peroxide can be removed by addition of 100 µL of sodium hypochlorite solution per liter. Another paper recommends using 5 mg/L Fe(II) and an equimolar amount of hydrogen peroxide at pH 5 for a 1 mg/L As(V) solution.[14] Discard the arsenic-containing precipitate.

B. *Using calcium hypochlorite and ferric sulfate*.[4] Mix 1.5 g ferric sulfate and 0.5 g calcium hypochlorite with 20 L water and allow it to react for 5–10 min. Filter this mixture through an 8 cm thick layer of fine sand at 0.5–2.0 L/min, test for completeness of decontamination, and discard it.

C. *Using a mixture of sand and iron filings*.[5] Pass the water through 3–4 filters containing a mixture of 2.5 g of iron filings and 100–150 g of sand at 1 L/h, test for completeness of decontamination, and discard it. Note that the geometry of the filters may influence the results and a final sand filter may be necessary. Dissolved oxygen is necessary for the procedure to work and the procedure appears to be more efficient at pH 6.[7]

D. *Using ferrous sulfate and calcium carbonate*.[10] Rapidly (500 rpm) stir an aqueous solution containing 50 mg/L As(V) with ferrous sulfate (Fe:As molar ratio 2:1) and calcium carbonate (Ca:Fe molar ratio 1.5:1) for 3 h. Discard the arsenic-containing sediment.

E. *Using titanium sulfate and UV irradiation*.[12] Prepare a solution containing 200 µg/L As(III), 1 mM sodium bicarbonate, and 1 mM sodium chloride, and adjust the pH to 5–6 with HCl or NaOH. Add 5–10 mg/L titanium sulfate and irradiate with a low-pressure UV lamp at 254 nm. Stir at 300 rpm for 60 s and at 150 rpm for 20 min. Discard the arsenic-containing precipitate.

F. *Degradation of diphenylarsinic acid with UV light*.[13] Irradiate a solution containing 5.40–7.06 mg/kg diphenylarsinic acid with a high-pressure mercury lamp for 30 min. The mixture of As(III) and As(V) oxides so formed should be further treated.

Analytical Procedures

As(III) and As(V) can be detected by ion chromatography with ICP-MS detection.[3] A 50 mm × 4 mm i.d. Dionex Ion Pac NG1 guard column and a 250 mm × 4 mm i.d. Dionex Ion Pac NS1 column were used with a mobile phase of isopropanol:1 mM tetrabutylammonium hydroxide (8:92). The detector was a VG-Plasma Quad 3 detector and ions *m*/*z* 75 and 77 were monitored. Retention times for As(III) and As(V) were ca. 120 and 250 s, respectively. Instrumental parameters are provided in the original paper.

For the analysis of diphenylarsinic acid, a 150 × 4.6 mm 5 µm Shimadzu VP-ODS C18 column was used with a mobile phase of MeCN:20 mM pH 2.7 potassium phosphate buffer flowing at 1 mL/min. the detector was set at 220 nm.[13]

Related Compounds

The procedure is specific for arsenic. Procedures have also been published for the immobilization of arsenic by the formation of calcium arsenate[15] and the immobilization of cacodylic acid (dimethylarsinic acid, $(CH_3)_2As(O)OH$) using potter's clay or hydraulic cement[16] or with starch paste.[17]Low-cost remediation procedures have been reviewed.[18, 19]

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AZIDES

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Sodium azide (Smite, Azium, Kazoe, NaN_3) [26628-22-8] can decompose explosively on heating and can form shock-sensitive and highly explosive azides when it comes in contact with heavy metals.[1] For this reason, solutions of NaN_3 should **never** be poured down the sink. Sodium azide is also incompatible with a number of other reagents, particularly halogenated solvents such as dichloromethane,[2–5] and it is acutely toxic[6] and may be carcinogenic.[7] A death has been reported when H_2O preserved with NaN_3 was ingested in the laboratory,[8] and it has been used in a possibly deliberate poisoning.[9] Sodium azide is a powerful mutagen. Treatment with acid liberates explosive, toxic, volatile (bp 37 °C) hydrazoic acid (HN_3).[10]

Organic azides vary greatly in stability, but a number of them are known to decompose explosively with shock or heating. For example, phenyl azide ($C_6H_5N_3$, azidobenzene) [622-37-7] explodes when heated and when mixed with Lewis acids, and benzyl azide ($C_6H_5CH_2N_3$) [622-79-7] is a heat-sensitive explosive.[11] Dimethyl-2-azidoethylamine[12] ($(CH_3)_2NCH_2CH_2N_3$) [86147-04-8] is a relatively new and experimental tertiary amine azide rocket fuel that has been proposed as an alternative to some of the hydrazines on account of its lower toxicity. These compounds should be handled very carefully.

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Table 1 Destruction of Azide in Buffer Solution With Nitrite[a]

Solvent	Final pH	$NaNO_2$ Required (g)	Molar Ratio NO_2^-/N_3^-	NaN_3 Left (%)
1 M HCl	1.2	0.25	4.7	<0.02
pH 3 buffer	4.2	0.25	4.6	<0.02
pH 7 buffer	7.1	2.5	45	<0.02
H_2O	8.0	5	95	<0.02
0.1 M Na_2CO_3	10.3	5	95	<0.02
1 M KOH	12.9	5	95	<0.02

[a] In each case, 50 mL of a 1 mg/mL solution of NaN_3 was stirred with the indicated amount of sodium nitrite for 18 h before analysis.

An explosion has been reported when diazidomethane formed in a reaction mixture containing dichloromethane and sodium azide.[13]

Principles of Destruction

Sodium azide can be oxidized by ceric ammonium nitrate[14] to nitrogen[15] or by nitrous acid[16] to nitrous oxide.[15] Some toxic nitrogen dioxide may be produced as a by-product of these reactions so they should always be done in a chemical fume hood. Destruction was >99.996%. Sodium azide in buffer solution may also be degraded by the addition of sodium nitrite.[17] The reaction proceeds much more readily at low pH, but if sufficient sodium nitrite is added, it will proceed to completion even at high pH (Table 1). At low pH, it may be possible to completely degrade the azide present in the buffer with less than the amount of sodium nitrite indicated below. However, the reaction mixture must be carefully checked to make sure that no azide remains. At high pH, it is possible for unreacted azide to remain in the presence of excess nitrite.

In an aqueous solution, sodium azide dissociates to hydrazoic acid (HN_3) and NaOH. Hydrazoic acid can be removed from solution using a strong anion-exchange resin.[18]

Organic azides (e.g., phenyl azide, PhN_3) can be reduced to the corresponding amines with tin in hydrochloric acid (HCl) or with stannous chloride in methanol. Dimethyl-2-azidoethylamine can be reduced to *N,N*-dimethylethylenediamine using nickel–aluminum alloy in sodium hydroxide solution.[12] The yield of *N,N*-dimethylethylenediamine was 94%, and the destruction of dimethyl-2-azidoethylamine was >99.9%.

Destruction Procedures

Sodium Azide

1. For each gram of NaN_3, stir 9 g of ceric ammonium nitrate in 30 mL of H_2O until it has dissolved. Dissolve the NaN_3 in 5 mL of H_2O and add it in 1-mL portions over 5 min. On a larger scale, an ice bath may be required for cooling. Stir the reaction mixture for 1 h, then check that it is still oxidizing. Add a few drops of the reaction mixture to an equal volume of 10% (w/v) potassium iodide (KI) solution, then

acidify with a drop of 1 M HCl and add a drop of starch solution as an indicator. The deep blue color of the starch–iodine complex indicates that excess oxidant is present. Analyze for completeness of destruction and discard the reaction mixture. If excess oxidant is not present, add more ceric ammonium nitrate.

2. Dissolve NaN_3 (5 g) in 100 mL of H_2O. Stir the reaction mixture and add 7.5 g of sodium nitrite dissolved in 38 mL of H_2O.[15] Slowly add dilute H_2SO_4 (4 M) until the reaction mixture is acidic to litmus. On a larger scale, an ice bath may be required for cooling. Stir for 1 h. Add a few drops of the reaction mixture to an equal volume of 10% (w/v) KI solution, then acidify with a drop of 1 M HCl and add a drop of starch solution as an indicator. The deep blue color of the starch–iodine complex indicates that excess nitrous acid is present. If excess nitrous acid is present, analyze for completeness of destruction and discard the reaction mixture. If excess nitrous acid is not present, add more sodium nitrite.

   Note: It is important to add the sodium nitrite, **then** the H_2SO_4. Adding these reagents in the reverse order will generate explosive, volatile, and toxic hydrazoic acid.

3. If necessary, dilute with water so that the concentration of sodium azide in the buffer solution does not exceed 1 mg/mL. For each 50 mL of buffer solution, add 5 g of sodium nitrite, stir the reaction for 18 h, check for completeness of destruction, and discard it.
4. Wash Dowex SAR (strong anion-exchange) resin several times with acetone and water, rinse with hexane several times, dry at 60 °C for at least 24 h, and allow it to cool in a desiccator. Pack a 16-mm i.d. × 200-mm long glass column with 10 g of the treated resin and pass an aqueous solution containing up to 600 µg/mL hydrazoic acid through at 10 mL/min. For a detection level of 0.1 µg/mL, the breakthrough volume is estimated to be 1178 mL. The column can be regenerated using 10% sodium chloride solution.

Organic Azides

1. Suspend 10.9 g of stannous chloride with stirring in 40 mL of methanol and add 4 g (0.03 mol) of benzyl azide dropwise.[19] When addition is complete, stir the reaction mixture at room temperature for 30 min, then dilute with H_2O, neutralize, and discard it.
2. Slowly add 1 g (0.0084 mol) phenyl azide to a stirred mixture of 6 g of granular tin in 100 mL of concentrated HCl.[20] Stir the mixture for 30 min after addition is complete, then pour it into a large quantity of cold H_2O, neutralize, and discard it. Unreacted tin may be recycled or discarded with solid waste.
3. Dilute an aqueous solution of dimethyl-2-azidoethylamine, if necessary, with H_2O so that the concentration does not exceed 10 mg/mL.[12] Add an equal volume of NaOH solution (1 M) and stir the mixture magnetically. For every 100 mL of this solution, add 5 g of Ni–Al alloy at such a rate that excessive frothing does not occur. The reaction can be quite exothermic. Do it in a reaction vessel whose volume is

at least three times that of the final reaction mixture. Cover the reaction mixture and stir for 24 h, then filter it through a pad of Celite®. Neutralize the filtrate, check for completeness of destruction, and discard it. Allow the spent nickel to dry on a metal tray for 24 h (away from flammable solvents) and discard it with the solid waste. Alternatively, hydrochloric acid may be cautiously added to the final reaction mixture to dissolve the solid nickel.

Analytical Procedures

Analysis for Sodium Azide

It is reported that hydrazoic acid can be determined by UV absorbance at 222 nm.[18] Sodium azide is analyzed by reacting azide ion with 3,5-dinitrobenzoyl chloride to form 3,5-dinitrobenzoyl azide, which can be determined by HPLC.[21] Prepare the following solutions: sulfamic acid (20% w/v in H_2O), sodium azide (100 µg/mL in H_2O), potassium hydroxide (KOH) (1 M), and HCl (0.2 M). To prepare the indicator, dissolve 0.1 g bromocresol purple in 18.5 mL 0.01 M KOH and make up to 25 mL with H_2O. The analysis was performed by reverse-phase HPLC with a mobile phase of water:acetonitrile (50:50) flowing at 1 mL/min. The UV detector was set at 254 nm, and the peak for 3,5-dinitrobenzoyl azide came at about 9 min. The limit of detection of sodium azide was 0.2 µg/mL. An analogous method has been described for determining trace amounts of azide ion in pharmaceutical substances by reacting azide ion with pentafluorobenzyl bromide to form pentafluorobenzyl azide, which can be determined by HPLC.[22] Add 100 µL of a 2% solution of pentafluorobenzyl bromide in DMSO to 5 mL of a 10 mg/mL solution of the substance to be tested in DMSO. After 6–16 h analyze by reverse-phase HPLC using a 150 × 4.6 mm 5 µm Phenomenex Luna phenyl-hexyl column at 40 °C, 6 µL injection, and detection at UV 210 nm. The mobile phase is a gradient with water:MeCN:trifluoroacetic acid from 90:10:0.005 to 20:80:0.005 over 30 min. Maintain at 20:80:0.005 for 5 min, return to initial conditions over 0.1 min, and re-equilibrate for 4.9 min. The flow rate is 1 mL/min. The peak for pentafluorobenzyl azide is at about 22.6 min. The limit of quantitation of sodium azide is 50 ppm. Note that the presence of water will interfere with the derivatization reaction by hydrolyzing the pentafluororbenzyl bromide.

1. *Analysis for azide in the presence of nitrite*. Remove a 5 mL aliquot of the reaction mixture and remove excess nitrite by adding at least 1 mL of the sulfamic acid solution. More sulfamic acid solution may be required for strongly basic reaction mixtures or those containing high concentrations of nitrite. [Complete destruction of nitrite can be checked using a modified Griess reagent (see below).] After standing for at least 3 min, the analytical solution may be spiked, if desired, by adding a small quantity of NaN_3 solution (ca. 10–20 µL). Add one drop of indicator and basify the mixture by adding KOH solution until it turns purple (typically, 3–10 mL are required). Add 2 mL of acetonitrile (4 mL if more than 1 mL of sulfamic acid was used), then add HCl dropwise until the mixture is

acidic (yellow color) followed by one more drop. Prepare a 10% (w/v) solution of 3,5-dinitrobenzoyl chloride in acetonitrile and add 50 µL. Shake the mixture and allow it to stand for at least 3 min before analysis by HPLC. Further standing times have no effect on the analyses. In this analytical procedure, the crucial part is the use of freshly prepared 3,5-dinitrobenzoyl chloride solution. This solution should be used within minutes of preparation. It is generally most convenient to prepare all the analytical samples (spiked and unspiked) with the fresh solution at the beginning of the day and then analyze them in the course of the day.

2. *Analysis for azide in the presence of ceric salts*. Remove a 10 mL aliquot of the reaction mixture and dilute with 40 mL of H_2O. Add 5 mL of this solution to 3 mL of 1 M KOH. (If <3 mL of 1 M KOH is used precipitation of ceric salts is not complete.) Shake and centrifuge the mixture. Remove 2 mL of the supernatant and add to 1 mL of acetonitrile. At this point, the analytical solution may be spiked, if desired, by adding a small quantity of sodium azide solution (10–20 µL). Add one drop of indicator and then add HCl dropwise, until the mixture is acidic (yellow color) followed by one more drop. Prepare a 10% (w/v) solution of 3,5-dinitrobenzoyl chloride in acetonitrile and add 50 µL. Shake the mixture and allow it to stand for at least 3 min before analysis by HPLC. Further standing times have no effect on the analyses. In this analytical procedure, the crucial part is the use of freshly prepared 3,5-dinitrobenzoyl chloride solution. This solution should be used within minutes of preparation. It is generally most convenient to prepare all the analytical samples (spiked and unspiked) with the fresh solution at the beginning of the day and then analyze them in the course of the day.

Analysis for Nitrite[23]

Boil 0.1g α-naphthylamine in 20 mL of H_2O until it dissolves. Pour this solution, while hot, into 150 mL of 15% (v/v) aqueous acetic acid. To this solution add a solution of 0.5 g of sulfanilic acid in 150 mL of 15% (v/v) aqueous acetic acid. Store the reagent in a brown bottle. Add 3 mL of the mixture to be tested to 1 mL of the reagent and allow it to stand at room temperature for 6 min. Measure the absorbance at 520 nm against a suitable blank. Limit of detection was 0.06 µg/mL of sodium nitrite. Note that at high pH, the reaction between azide and nitrite is quite slow so that the presence of excess nitrite does not mean that all the azide has been degraded. α-Naphthylamine may be a carcinogen.[24, 25] A procedure using *N*-(1-naphthyl)ethylenediamine dihydrochloride instead has been described more recently.[26]

Related Compounds and Reactions

The procedures described above for NaN_3 should not be used for heavy metal azides, many of which are shock-sensitive explosives. Professional help should be sought in these cases. The procedures for organic azides should be generally applicable, although in all cases, the reactions should be thoroughly validated to ensure that the azides are completely destroyed. The products of these reactions are the

corresponding amines, which may themselves be hazardous compounds. It has been reported that, although dimethyl-2-azidoethylamine can be satisfactorily reduced to *N*,*N*-dimethylethylenediamine using nickel–aluminum alloy in NaOH solution or tin in hydrochloric acid, the product when stannous chloride in methanol was used was the possibly toxic 2-chloroethyldimethylamine.[12]

It should be noted that sodium hypochlorite is not recommended for the destruction of sodium azide due to the formation of the toxic intermediate chlorine azide under acidic conditions and slow kinetics under basic conditions.[27]

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AZO AND AZOXY COMPOUNDS AND TETRAZENES

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Azobenzene (**I**, R = R′ = H, mp 68 °C) [103-33-3],[1] azoxybenzene (**II**, R = R′ = H, mp 36 °C) [495-48-7],[2] *N,N*-dimethyl-4-amino-4′-hydroxyazobenzene [**I**, R = $N(CH_3)_2$, R′ = OH, mp 201–202 °C] [2496-15-3], azoxyanisole (**II**, R = R′ = OCH_3, 4,4′-dimethoxyazoxybenzene, mp 118 °C) [13620-57-0], phenylazophenol (**I**, R = OH, R′ = H, mp 150–152 °C) [1689-82-3],[3] phenylazoaniline (**I**, R = NH_2, R′ = H, mp 123–126 °C) [60-09-3],[4] and fast garnet (**III**, mp 101–102 °C) [97-56-3][5] are solids and azoxymethane (AOM, CH_3-N=N(O)-CH_3, bp 98 °C) [25843-45-2] and tetramethyltetrazene [$(CH_3)_2$N-N=N-N$(CH_3)_2$, bp 130 °C] [6130-87-6] are liquids. Generally, these compounds are only slightly soluble in H_2O (5–50 ppm), but they are soluble in alcohols and organic solvents. Azoxymethane,[6] phenylazoaniline,[7] and fast garnet[8] are carcinogenic in animals and azobenzene may be carcinogenic in animals.[9, 10] Azoxybenzene is toxic by ingestion and is a skin and eye irritant;[11] tetramethyltetrazene explodes when heated to its boiling point.[12] The mutagenicity of azo dyes has been reviewed.[13] Azoxymethane is used in cancer research, and the other compounds are used in organic synthesis and as intermediates in the chemical industry.

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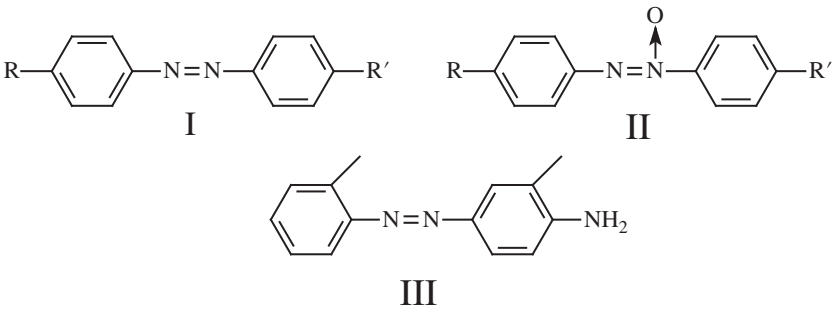

Principles of Destruction

All of the compounds except azoxybenzene (destruction was incomplete) and azoxymethane and tetramethyltetrazene (not tested) can be oxidized by potassium permanganate in sulfuric acid ($KMnO_4$ in H_2SO_4). Destruction is >99.5%, and the products have not been identified.[14, 15] The other compounds can be reduced to their parent amines using nickel–aluminum (Ni–Al) alloy in potassium hydroxide (KOH) solution.[16] Destruction is >99% for tetramethyltetrazene and >99.5% for the other compounds.[15] When performing these reactions, it should be noted that in some cases, the products are aromatic amines, which may also be hazardous. For example, the product of the reduction of azobenzene and azoxybenzene is aniline for which limited evidence of carcinogenicity in animals exists.[17] In fact, this procedure is not recommended for phenylazoaniline or fast garnet because the final reaction mixtures and *p*-phenylenediamine (from phenylazoaniline) and 2,5-diaminotoluene (from fast garnet) are mutagenic. Balakrishnan et al. have pointed out that reduction of azo compounds with iron may result in the generation of toxic aromatic amines.[18]

Destruction Procedures

Destruction of Azobenzene, Azoxybenzene, Azoxyanisole, Phenylazophenol, Azoxymethane, and Tetramethyltetrazene

Dissolve the compound in H_2O (azoxymethane and tetramethyltetrazene) or methanol (others) so that the concentration does not exceed 5 mg/mL and add an equal volume of 2 M KOH solution. For each 100 mL of basified solution, add 5 g of Ni–Al alloy. Add quantities of more than 5 g in portions over approximately 1 h to avoid excessive frothing. Stir the reaction mixture overnight, then filter through a pad of Celite®. Neutralize the filtrate, test for completeness of destruction, and discard it. Place the nickel that is filtered off on a metal tray away from flammable solvents for 24 h, then discard it with the solid waste (Table 1).

Table 1 Destruction of Azo and Azoxy Compounds Using Nickel–Aluminum Alloy in Potassium Hydroxide Solution

Compound	% Remaining	Mutagenicity of Reaction Mixture[a]	Products (%)
Azobenzene	<0.26	–	Aniline (84)
Azoxybenzene	<0.12	–	Aniline (95)
Azoxyanisole	<0.22	–	*p*-Anisidine (69)
Phenylazophenol	<0.38	–	Aniline (99); *p*-aminophenol (34)
Phenylazoaniline	<0.14	+	Aniline (93); *p*-phenylenediamine (81)
Fast Garnet	<0.05	+	*o*-Toluidine (92); 2,5-diaminotoluene (98)
Azoxymethane	<0.5	nt	Methylamine (100)
Tetramethyltetrazene	<1	nt	Dimethylamine (94)

[a] – = nonmutagenic; + = mutagenic; nt = not tested.

Table 2 Destruction of Azo and Azoxy Compounds Using Potassium Permanganate in Sulfuric Acid

Compound	% Remaining	Mutagenicity of Reaction Mixture[a]
Azobenzene	<0.13	–
Azoxybenzene	0.46	–
Azoxyanisole	<0.17	–
Phenylazophenol	<0.033	–
Phenylazoaniline	<0.07	–
Fast garnet	<0.063	–

[a] – = nonmutagenic.

Destruction of Azobenzene, Azoxyanisole, Phenylazophenol, Phenylazoaniline, and Fast Garnet

Prepare a 0.3 M solution of $KMnO_4$ in 3 M H_2SO_4 by stirring 47.4 g of $KMnO_4$ per liter of 3 M H_2SO_4 for at least 15 minutes but no more than 1 h. Take up the compound in glacial acetic acid so that the concentration does not exceed 10 mg/mL (5 mg/mL for phenylazoaniline) and add 40 mL (80 mL for phenylazoaniline) of the $KMnO_4/H_2SO_4$ mixture for each 1 mL of this solution. After 18 h, decolorize with sodium metabisulfite, make strongly basic with 10 M KOH solution (**Caution!** Exothermic!), dilute with H_2O, filter to remove manganese compounds,[19] test the filtrate for completeness of destruction, neutralize, and discard it (Table 2).

Destruction of N,N-Dimethyl-4-Amino-4′-Hydroxyazobenzene

Take up 0.24 mg of *N,N*-dimethyl-4-amino-4′-hydroxyazobenzene in 1 mL of 50% (v/v) acetic acid and add 1 mL of 2 M H_2SO_4 and 1 mL of 0.2 M $KMnO_4$ solution. After 2 h, decolorize with oxalic acid, test for completeness of destruction, neutralize, and discard it.

Analytical Procedures

Basify 20 mL aliquots of the $KMnO_4$ reaction mixtures with 20 mL 10 M KOH solution, dilute with 50 mL water, cool, and extract three times with 10-mL portions of cyclohexane. Dry the extracts over anhydrous sodium sulfate, evaporate, and take up the residue in about 2 mL of methanol. Analyze this solution by HPLC for the presence of the starting material.

Filter 20 mL aliquots of the Ni–Al alloy reaction mixtures through a pad of Celite® with 50 mL of cyclohexane. Separate the layers and extract the aqueous layer three times with 10 mL portions of cyclohexane. Combine all the organic layers, dry over anhydrous sodium sulfate, evaporate, and take up the residue in about 2 mL of methanol. Analyze this solution by HPLC for the presence of the starting material. Nickel–aluminum alloy reaction mixtures can also be analyzed directly by GC.

Because of the presence of the phenolic group, the procedures for phenylazophenol were a little different. The $KMnO_4$ reaction mixture was not basified before extraction, and the Ni–Al alloy reaction mixture was processed as follows. A 20 mL aliquot was filtered through a pad of Celite® with 50 mL of cyclohexane and the layers of the filtrate were separated. The aqueous layer was extracted once with 20 mL of cyclohexane, then acidified with 2 mL of 6 M H_2SO_4 and extracted five times with 20 mL portions of cyclohexane. All the organic layers were combined and dried over anhydrous sodium sulfate, evaporated, and the residue taken up in about 2 mL of methanol.

In all cases, control experiments were performed to ascertain that the recoveries of the starting materials were satisfactory.

Some of the starting materials were determined by HPLC using a 250 mm × 4.6 mm i.d. column of Microsorb C8 (Varian Inc., Palo Alto, CA) with methanol–water mixtures flowing at 1 mL/min as the mobile phases. The UV detector was set at 254 nm. The methanol:water ratios, with retention times in minutes in parentheses, were azobenzene 70:30 (13.9), azoxybenzene 70:30 (12.3), azoxyanisole 70:30 (14.3), phenylazophenol 60:40 (16.9), phenylazoaniline 60:40 (12.4), and fast garnet 70:30 (11.4). *p*-Aminophenol was also determined by HPLC using methanol:20 g/L ammonium acetate solution 7:93 as the mobile phase.[20] An aliquot of the centrifuged reaction mixture was acidified with an equal volume of 2 M HCl, neutralized with sodium bicarbonate, centrifuged, and an aliquot of the supernatant diluted 10-fold with 20 g/L ammonium acetate solution. The retention time was 6.9 min.

Some of the starting materials and products were determined by GC, using a 1.8 m × 2 mm i.d. packed column with flame ionization detection.[15] For methylamine (retention time 0.9 min at 60 °C), dimethylamine (1.3 min at 100 °C), azoxymethane (2.6 min at 120 °C), and tetramethyltetrazene (3.4 min at 150 °C), the packing was 28% Pennwalt 223 + 4% KOH on 80/100 Gas Chrom R. For aniline (2.9 min at 80 °C), azobenzene (1.5 min at 180 °C), and azoxybenzene (3.2 min at 180 °C), the packing was 3% SP 2401-DB on 100/120 Supelcoport. For *p*-anisidine (2.7 min at 100 °C), *p*-phenylenediamine (1.2 min at 130 °C), *o*-toluidine (2.7 min at 80 °C), and 2,5-diaminotoluene (1.7 min at 130 °C), the packing was 3% OV1 on 80/100

Supelcoport. These chromatographic conditions are only a guide; the exact conditions would have to be determined experimentally.

N,N-Dimethyl-4-amino-4′-hydroxyazobenzene was determined by differential pulse polarography of the reaction mixture.[14]

A liquid chromatography-mass spectrometry method has been described for the analysis of aromatic amines obtained after azo dyes are reduced with sodium dithionite.[21] However, the efficiency of the reduction process was not determined.

Mutagenicity Assays

Reaction mixtures from the degradation of azobenzene, azoxybenzene, azoxyanisole, phenylazophenol, phenylazoaniline, and fast garnet were tested for mutagenicity as described on page 4. Tester strains TA98, TA100, TA1530, and TA1535 were used. Before testing $KMnO_4$ reaction, mixtures were decolorized with sodium ascorbate, then neutralized with sodium bicarbonate and Ni–Al alloy reaction mixtures were centrifuged and neutralized with acetic acid. The only reaction mixtures that were mutagenic were those obtained when phenylazoaniline and fast garnet were degraded with Ni–Al alloy. This is probably because the products of these reactions, *p*-phenylenediamine and 2,5-diaminotoluene, were mutagenic. Azobenzene, azoxybenzene, phenylazophenol, phenylazoaniline, and fast garnet were all mutagenic when tested as DMSO solutions.

Related Compounds

Nickel–aluminum alloy in KOH solution appears to be a general method for the cleavage of N—N and N—O bonds[16] and so it should be applicable to other azo and azoxy compounds. Potassium permanganate in H_2SO_4 is a general oxidative procedure, and it should be applicable to many azo compounds.[14] In each case, full validation should be carried out before using the procedure.

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2. Other names for this compound are diphenyldiazene 1-oxide, azobenzene oxide, azoxybenzide, and azoxydibenzene.
3. Other names for this compound are 4-hydroxyazobenzene, Solvent Yellow 7, p-benzeneazophenol, C.I. Solvent Yellow 7, C.I. 11800, and 4-phenylazophenol.
4. Other names for this compound are 4-aminoazobenzene, 4-(phenylazo)benzenamine, C.I. Solvent Yellow I, p-aminodiphenylimide, aniline yellow, C.I. 11000, AAB, 4-aminoazobenzol, 4-benzeneazoaniline, Brasilazina Oil Yellow G, Ceres Yellow R, C.I. Solvent Blue 7, Fast Spirit Yellow AAB, Oil Soluble Aniline Yellow, Oil Yellow AAB, Organol Yellow, paraphenolazo aniline, p-phenylazophenylamine, Sudan Yellow R, and USAF EK-1375.
5. Other names for this compound are C.I. 11160, 4′-amino-2,3′-dimethylazobenzene, o-aminoazotoluene, Solvent Yellow 3, 2-amino-5-azotoluene, AAT, o-AAT, o-aminoazotoluol, o-AT, Brasilazina Oil Yellow R, Butter Yellow, C.I. 11160, C.I. 11160B, C.I. Solvent Yellow 3, 2′,3-dimethyl-4-aminoazobenzene,

Fast Garnet GBC Base, Fast Oil Yellow, Fast Yellow AT, Fast Yellow B, Hidaco Oil Yellow, 2-methyl-4-[(2-methylphenyl)azo]benzenamine, OAAT, Oil Yellow, Oil Yellow 21, Oil Yellow 2681, Oil Yellow AT, Oil Yellow A, Oil Yellow C, Oil Yellow I, Oil Yellow 2R, Oil Yellow T, Organol Yellow 25, Somalia Yellow R, Sudan Yellow RRA, o-tolueneazo-o-toluidine, 5-(o-tolylazo)-2-aminotoluene, 4-(o-tolylazo)-o-toluidine, Tulabase Fast Garnet GB, Tulabase Fast Garnet GBC, and Waxacol Yellow NL.

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BORON TRIFLUORIDE AND INORGANIC FLUORIDES

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Soluble inorganic fluorides are toxic[1] (e.g., the LD_{50} of sodium fluoride (NaF) [7681-49-4] is 180 mg/kg).[2] Boron trifluoride (BF_3) is highly toxic,[3] and when supplied as the etherate ($BF_3 \cdot Et_2O$) [109-63-7], it is corrosive and flammable.[4] These compounds are used industrially and in chemistry laboratories. Potassium fluoride (KF) [7789-23-3][5] and NaF[6] are teratogens, ammonium hydrogen difluoride[7] [$(NH_4)HF_2$] [1341-49-7] is corrosive, and sodium hexafluorosilicate (Na_2SiF_6) [16893-85-9][8] is a skin and severe eye irritant. Sodium fluoride, KF, and Na_2SiF_6 are used as pesticides. Tin(II) fluoride (SnF_2) [7783-47-3] is used as a dental caries prophylactic.

Principles of Destruction and Decontamination

The compound $BF_3.Et_2O$[9] is very rapidly hydrolyzed by H_2O to boric acid and inorganic fluoride.[3, 10] Addition of a calcium salt will produce insoluble (solubility 16 mg/L), nontoxic ($LD_{50} > 2.5$ g/kg),[11] calcium fluoride (CaF_2), which can easily be removed by filtration. In a similar fashion, addition of calcium ions to 10 mg/mL solutions of KF, NaF,[12] $(NH_4)HF_2$,[13] and SnF_2[14] or a 5 mg/mL solution of Na_2SiF_6[15] removed fluoride from solution. A variety of different procedures was tried, and it was found that the best

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Table 1 Removal of Fluoride from Solution Using the Destruction and Decontamination Procedures Below

	Compound					
	BF_3	KF	NaF	$(NH_4)HF_2$	SnF_2	Na_2SiF_6
Initial concentration (mg/mL)	11.54	10	10	10	10	5
Initial concentration (ppm F^-)	4632	3276	4524	6667	2425	3032
Final concentration (ppm F^-)	5.2	13.5	27	8	3.7	14.5

results were obtained when calcium oxide (CaO) was used.[10] It was found in control experiments that CaF_2 is more soluble at low pH. Calcium oxide may keep the pH high and thus decrease the solubility of CaF_2, and hence, the final fluoride concentration. When CaO was used, the final fluoride concentration was <30 ppm in each case (Table 1). It was found advisable to allow the reaction mixture to settle, and then to carefully decant the clear liquid into the filter funnel. Finally, the residual sludge was filtered. This procedure helped to prevent clogging of the filter paper.

Destruction and Decontamination Procedures[10]

Destruction of Boron Trifluoride Etherate

Stir 100 mL of H_2O and 2.5 g of CaO, and add 1 mL of $BF_3{\cdot}Et_2O$. Stir the mixture for 18 h, allow it to settle, filter, check for completeness of decontamination, remove any organic liquid that is present, and discard it.

Decontamination of Solutions Containing Fluoride

If necessary, dilute with H_2O so that the concentration of KF, NaF, $(NH_4)HF_2$, or SnF_2 does not exceed 10 mg/mL, and the concentration of Na_2SiF_6 does not exceed 5 mg/mL. For each 10 mL of solution, add 0.5 g of CaO, stir for 18 h, allow it to settle, filter, check for completeness of decontamination, and discard it.

Analytical Procedures

Mix 9 mL of H_2O, 1 mL of a total ion strength adjustment buffer (TISAB III), and 1 mL of sample and determine the fluoride concentration using a fluoride combination ion-selective electrode (Orion 960900 or similar). Calibrate the electrode using solutions containing known concentrations of fluoride ions. Create a calibration curve by graphing the reading (mV) against the concentration of fluoride (ppm) on semilog graph paper. Alternatively, meters can be purchased that can be programmed to display the ion concentration directly.

Related Compounds

This procedure should be generally applicable to other inorganic fluorides and other complexes of BF_3. However, it was found that the procedure cannot be used for disodium fluorophosphate because the fluoride is not completely ionized in solution.

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13. Other names for this compound are ammonium bifluoride, acid ammonium fluoride, and ammonium hydrogen fluoride.
14. Other names for this compound are stannous fluoride, tin difluoride, Fluoristan, and tin bifluoride.
15. Other names for this compound are sodium fluosilicate, sodium silicofluoride, Salufer, disodium hexafluorosilicate, Destruxol Applex, disodium silicofluoride, Ens-Zem Weevil Bait, ENT 1,501, Ortho Earwig Bait, Ortho Weevil Bait, Prodan, PSC Co-op Weevil Bait, Safsan, silicon sodium fluoride, sodium fluorosilicate, sodium hexafluorosilicate, and Super Prodan.

BOTULINUM TOXINS

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

The *Clostridium botulinum* organism, a spore-forming bacillus, produces potent neurotoxins of seven serologically different types: A [93384-43-1]; B [93384-44-2]; C [93384-45-3]; D [93384-46-4]; E [93384-47-5]; F [107231-15-2]; and G [107231-16-3]. These toxins are acutely lethal and are active by inhalation, ingestion,[1] and injection.[2, 3]

There are no currently approved vaccines for botulinum neurotoxins, but in 2013, FDA approved an antitoxin for the treatment of botulism.[4] Candidate vaccines are in clinical trials.[5]

The botulinum neurotoxins have been identified as select agent toxins.[6] Guidelines for working with the select agent toxins have been published by the Centers for Disease Control and Prevention (CDC).[7]

Principles of Destruction

The botulinum neurotoxins can be inactivated by steam autoclaving, heat, sodium hypochlorite solutions, and sodium hydroxide solutions. The method of choice will depend on the matrix in which the neurotoxin is found. Inactivation methods have not

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been validated for all neurotoxin serotypes; validation should be established using specific toxin bioassays.

Other approaches to inactivation have been investigated: ozone, powdered activated charcoal, and coagulation with ferric chloride,[8] and chloramines, iodine, and chlorine dioxide[9] have been used. While in some cases and in some circumstances, these approaches were successful, their application in the laboratory setting is limited.

Note: Previous editions of this book have described methods using sodium hypochlorite for the degradation of various compounds. However, it has become increasingly apparent that the use of sodium hypochlorite leads, in many cases, to the generation of toxic products. See the monograph on sodium hypochlorite near the beginning of this book. We recommend that a risk–benefit analysis be carried out before continuing to employ sodium hypochlorite for this degradation procedure.

Destruction Procedures

Inactivation by Steam Autoclave

Autoclaving for 1 h at 121 °C ensures that sufficient heat has penetrated for complete toxin inactivation and that all spores present are killed. Volumes larger than 1 L should be autoclaved for 2 h.[10]

Inactivation by Application of Heat

Heating to an internal temperature of 85 °C for more than 5 min has been recommended for the inactivation of the toxin.[11, 12] Because heat denaturation of the neurotoxins is biphasic as a function of time, the CDC has recommended heating to 100 °C for 10 min for inactivation.[13]

Inactivation Using Sodium Hypochlorite Solutions

Solutions of NaOCl at or above concentrations of 0.1% (see below for assay procedure) for 30 min will inactivate botulinum neurotoxins.[8, 9, 12] This technique is also recommended for decontaminating work surfaces and spills[14] (see also below).

Treatment of Spills Using Sodium Hydroxide Solutions

Confine the spill with absorbent paper and wet the paper with at least five times the spill volume of 0.1 N NaOH. After a few minutes, remove the absorbent paper for autoclaving. Work surfaces may also be treated with NaOH.[10]

Detection Procedures

The analytical techniques for the detection of bacterial toxins,[15] including for botulinum neurotoxins, in particular, the mouse lethality test, standard immunological assays, polymerase chain reaction (PCR), and PCR in conjunction with immunological assays,[16] have been reviewed.

Assay of Sodium Hypochlorite Solution

Sodium hypochlorite solutions tend to deteriorate with time, so they should be periodically checked for the amount of active chlorine they contain. Pipette 10 mL of the NaOCl solution into a 100 mL volumetric flask and fill it to the mark with distilled H_2O. Pipette 10 mL of this solution into a conical flask containing 50 mL of distilled H_2O, 1 g of potassium iodide (KI), and 12.5 mL of 2 M acetic acid. Titrate this solution against a 0.1 N sodium thiosulfate solution using starch as an indicator. Each 1 mL of the sodium thiosulfate solution corresponds to 3.545 mg of active chlorine. Commercially available NaOCl solution (Clorox bleach) contains 5.25% NaOCl and should contain 45–50 g of active chlorine per liter.

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BREVETOXINS

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Brevetoxins [98225-48-0] are a suite of cyclic compounds based on either a 10- or 11-ring polyether backbone.[1] They are marine neurotoxins produced by the dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*, *Ptychodiscus brevis*), which bind to voltage-gated sodium channels. Respiratory exposure in humans may lead to neurotoxic shellfish poisoning. In general, the brevetoxins are acutely toxic by several routes of administration[2]; they are used in neurochemical research.[3] Brevetoxin-1 (PbTx-1, brevetoxin A, GB-1) [98112-41-5] has been shown to be teratogenic.[4] Brevetoxin-2 (PbTx-2, brevetoxin B, GB-2, T-34, T-47 [79580-28-2]) is soluble in acetone and chloroform, less soluble in ethyl acetate, methanol, and ethanol, and slightly soluble in water.[5] The structure of brevetoxin-2 is shown. Other brevetoxins have similar structures.

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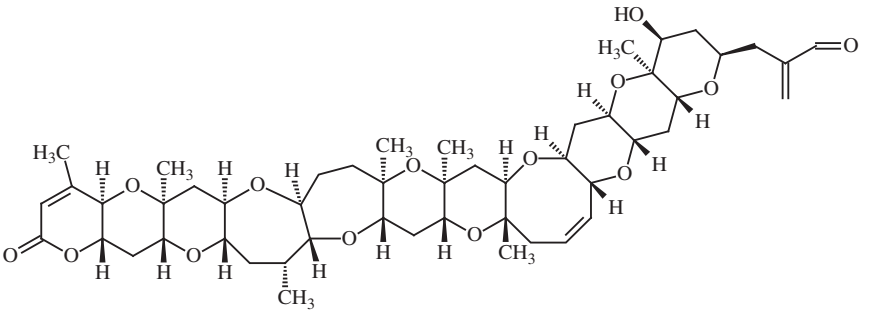

Although the brevetoxins are not currently identified as select agent toxins,[6] some institutions treat them as if they were. Guidelines for working with the select agent toxins have been published by the CDC.[7]

Principles of Destruction

Brevetoxin may be degraded using dilute sodium hypochlorite (NaOCl) solution and possibly using a combination of NaOCl and sodium hydroxide (NaOH). Degradation efficiency was about 99% in the former case; the efficiency of degradation in the latter case has not been determined.

Note: Previous editions of this book have described methods using sodium hypochlorite for the degradation of various compounds. However, it has become increasingly apparent that the use of sodium hypochlorite leads, in many cases, to the generation of toxic products. See the monograph on sodium hypochlorite near the beginning of this book. We recommend that a risk–benefit analysis be carried out before continuing to employ sodium hypochlorite for this degradation procedure.

Destruction Procedures

Inactivation Using Sodium Hypochlorite

Add 9 mL of a 1% NaOCl solution (see below for assay procedure) for each mL of toxin solution and let stand for 30 min. Using an assay based on mouse lethality, this procedure reduced the brevetoxin toxicity of the solution by about 2 orders of magnitude.[8] The products of the reaction have not been identified. The reaction mixtures resulting from this procedure have not been systematically tested for residual biological activity.

This procedure was also recommended for the inactivation of brevetoxin on equipment, animal cages, and spills.[8] In another study,[9] 10 min exposures to 0.1 N NaOH reduced the toxicity of brevetoxin-2, based on a fish lethality assay, by at least 1 order of magnitude. This approach was recommended for the decontamination of laboratory equipment, glassware, benchtops, and floors.

Inactivation Using Sodium Hypochlorite and Sodium Hydroxide

Treatment with NaOCl (see below for assay procedure) plus NaOH has been cited as a means of degrading brevetoxin.[10] This procedure does not appear to have been explicitly

tested in the original reference,[8] in which the combination of NaOCl and NaOH was used for the treatment of T-2 mycotoxin. Before employing this procedure, the extent to which it leads to the destruction of brevetoxin should be determined to see if it is adequate. It is also desirable to identify the products of the reaction and to test the reaction mixtures for residual activity.

Analytical Procedures

A HPLC technique for the separation of brevetoxins, coupled with electrospray ionization mass spectroscopy for their identification, has been described.[11, 12] A 100×1-mm i.d. reversed-phase C_{18} 3-μm Spherisorb column and UV detector at 215 nm were used. The mobile phase was methanol:water (85:15) at flow rates of from 2 to 30 μL/min; optimal results were obtained at a flow rate of about 4 μL/min. Another publication describes a liquid chromatography-mass spectrometry (LC-MS) method with solid-phase extraction used for sample preparation.[13] The limits of detection were 0.05 mg/kg or lower.

Assay of Sodium Hypochlorite Solution

Sodium hypochlorite solutions tend to deteriorate with time, so they should be periodically checked for the amount of active chlorine they contain. Pipette 10 mL of the NaOCl solution into a 100 mL volumetric flask and fill it to the mark with distilled H_2O. Pipette 10 mL of this solution into a conical flask containing 50 mL of distilled H_2O, 1 g of potassium iodide (KI), and 12.5 mL of 2 M acetic acid. Titrate this solution against a 0.1 N sodium thiosulfate solution using starch as an indicator. Each 1 mL of the sodium thiosulfate solution corresponds to 3.545 mg of active chlorine. Commercially available NaOCl solution (Clorox bleach) contains 5.25% NaOCl and should contain 45–50 g of active chlorine per liter.

Related Compounds

The destruction procedures described above were carried out on brevetoxin-2, but they should also be applicable to other brevetoxins. The procedure should be thoroughly validated in each instance to ensure that the compound has been completely destroyed as some variability may be experienced.

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BUTYLLITHIUM

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Butyllithium (*n*-butyl lithium) [109-72-8] is generally supplied as a solution in a hydrocarbon solvent (e.g., pentane, hexanes, or cyclohexane). Solutions will ignite on contact with H_2O and carbon dioxide (CO_2), and solutions of >20% will ignite spontaneously in the air.[1, 2] This compound should be handled under nitrogen with special equipment.[3] It is used in organic synthesis.

Principle of Destruction

Butyllithium is allowed to react with 1-butanol in a dry hydrocarbon solvent to give lithium butoxide and butane. The lithium butoxide is subsequently hydrolyzed to butanol and lithium hydroxide.[4] 1,10-Phenanthroline is used as an indicator. In the presence of excess lithium, red color is produced.[5] Alternatively, butyllithium can be reacted with a siloxane polymer to give alkylsilyl groups and lithium silanoate groups. The lithium silanoate is subsequently hydrolyzed with H_2O to lithium hydroxide and silanol groups.[6]

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Destruction Procedures

Caution! These reactions should be done under nitrogen and provision should be made for venting, through a bubbler, the considerable amounts of gas that may be generated.

1. Prepare a 10% (v/v) solution of 1-butanol in isooctane (2,2,4-trimethylpentane) and add 1,10-phenanthroline (1 mg/mL) as an indicator. Dry this solution over a 4 Å molecular sieve overnight. Stir 15 mL of the 1-butanol-isooctane mixture under nitrogen in an ice bath and cautiously add 5 mL of a 1.55 M butyllithium solution in hexanes. After 10 min, or when the reaction appears to have stopped, check the color of the mixture. If it is red, indicating the presence of excess butyllithium, add more of the 1-butanol-isooctane mixture until yellow color is produced. When the reaction mixture is yellow add 10 mL of H_2O. Stir the mixture overnight, separate the layers, and discard them.[4]
2. Prepare a mixture of equal volumes of poly(dimethylsiloxane) 200 fluid (5 centistokes, Aldrich 31,766-7 or equivalent) and dry THF that contains 2.5 mg/mL of 1,10-phenanthroline as an indicator. Stir 4 mL of the poly(dimethylsiloxane)–THF mixture under nitrogen and cautiously add 10 mL of a 1.55 M butyllithium solution in hexanes. After 3 h or when the color of the solution has turned from red to yellow, add 25 mL of H_2O. If the color does not change from red to yellow, add more of the polydimethylsiloxane–THF mixture. Stir the mixture for 1 h, separate the layers, and discard them.[6]

Analytical Procedures for Alkyllithium Reagents

Instead of destroying aged samples of butyllithium of uncertain titer, it may be more efficient to retitrate the reagent. Methods of analysis[7] and indicators[5] have been reviewed. Lithium reagents can be titrated with 2-butanol (*sec*-butyl alcohol) using 1,10-phenanthroline,[8] 2,2′-biquinoline,[8] or *N*-phenyl-1-naphthylamine,[9] which form colored complexes when alkyllithiums are present. When one equivalent of 2-butanol is added, the reaction mixture becomes colorless. Alternatively, alkyllithiums can be titrated with diphenylacetic acid,[10] 2,5-dimethoxybenzyl alcohol,[11] 1,3-diphenyl-2-propanone tosylhydrazone,[12] or 4-biphenylmethanol.[13] In each case, adding one equivalent of alkyllithium produces the colorless anion, and adding a slight excess produces the colored dianion.

Related Compounds

These techniques should be generally applicable to other alkyllithium reagents. The original reference[6] states that the poly(dimethylsiloxane) technique can also be used for methyllithium [917-54-4] and *tert*-butyllithium. Note that *tert*-butyllithium is considerably more pyrophoric than *n*-butyllithium. A death occurred following a laboratory accident with *tert*-butyllithium[14, 15] and the material should be treated with the utmost respect. Procedures for the safe handling of pyrophoric reagents such as these

have been described.[16] For disposal, the authors recommend adding these materials to an inert solvent and cooling the mixture in dry ice/acetone. Isopropanol is then slowly added until no further heat is released. Methanol is then added followed by copious amounts of water.

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CALCIUM CARBIDE

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Calcium carbide (acetylenogen, calcium acetylide, or CaC_2) [75-20-7] is used in the laboratory to generate acetylene. Calcium carbide reacts with small quantities of H_2O to generate acetylene and when this is done in an uncontrolled fashion explosive acetylene–air mixtures may be formed. Decomposition under controlled conditions generates acetylene, which is vented into the fume hood and calcium salts. With solutions of silver and copper salts, the corresponding explosive silver and copper acetylides are formed. Calcium carbide reacts vigorously with methanol after an induction period; it is incompatible with a variety of reagents including hydrogen chloride, iron(III) chloride, iron(III) oxide, lead difluoride, magnesium, and sodium peroxide.[1, 2]

Destruction Procedures

1. Slowly add, in small portions, 5 g of CaC_2 to 250 mL of H_2O stirred in a flask in a fume hood. When the reaction has ceased, neutralize the aqueous solution, and discard it.[3]

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2. Place CaC_2 (50 g) in a 2-L flask and suspend it by stirring in 600 mL of toluene or cyclohexane. Surround the flask with an ice bath and pass nitrogen into the flask. Exhaust the acetylene that is generated through a plastic tube into the back of the fume hood. Add hydrochloric acid (6 M, 300 mL) dropwise from a dropping funnel over about 5 h. Stir the mixture for another hour, then neutralize the aqueous layer, separate the layers, and discard them.[4]

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CARBAMIC ACID ESTERS

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Four carbamic acid esters were investigated: methyl carbamate [598-55-0] (MC, $CH_3OC(O)NH_2$);[1] ethyl carbamate,[2] which is more commonly called urethane [51-79-6] [UT, $CH_3CH_2OC(O)NH_2$]; *N*-methylurethane [105-40-8] [MUT, $CH_3CH_2OC(O)NHCH_3$]; and *N*-ethylurethane [623-78-9] [EUT, $CH_3CH_2OC(O)NHCH_2CH_3$]. Methyl carbamate (mp 56–58 °C) and urethane (mp 48.5–50 °C) are solids and MUT (bp 170 °C) and EUT (bp 176 °C) are liquids. However, liquefied methyl carbamate (bp 176–177 °C) and urethane (bp 182–184 °C) have fairly low boiling points, so all these compounds should be regarded as volatile and should only be handled inside a chemical fume hood. Urethane,[3] MUT,[4] and EUT[4] are carcinogenic in experimental animals. Evidence for the carcinogenicity of methyl carbamate is inconclusive.[5] Urethane is a teratogen that causes depression of bone marrow and nausea and can affect the brain and central nervous system.[6] Urethane is used industrially as an intermediate and it also appears to form naturally by fermentation in some alcoholic beverages. Methyl carbamate is used industrially in the textile and pharmaceutical industries. All of these compounds are soluble in H_2O and ethanol.

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Table 1 Summary of Reaction Conditions for the Hydrolysis of RNHC(O)OR′

Compound	Conditions	Time (h)	RNH_2 (%)	R′OH (%)	Amount Remaining (%)
MC	Room temperature	24	—	98	<0.61
	Reflux	4	—	75	<0.61
UT	Room temperature	24	—	103	<0.15
	Reflux	4	—	51	<0.16
MUT	Room temperature	48	60	92	<0.075
	Reflux	4	39	69	<0.075
EUT	Reflux	4	43	74	<0.15

Principles of Destruction

These compounds are all hydrolyzed using 5 M sodium hydroxide (NaOH) solution,[7] although the reaction times vary. Methyl carbamate is hydrolyzed to methanol and carbamic acid, and UT is hydrolyzed to ethanol and carbamic acid. MUT is hydrolyzed to ethanol and *N*-methylcarbamic acid and EUT is hydrolyzed to ethanol and *N*-ethylcarbamic acid. Carbamic acid decomposes to carbon dioxide (CO_2) and ammonia, *N*-methylcarbamic acid decomposes to CO_2 and methylamine, and *N*-ethylcarbamic acid decomposes to CO_2 and ethylamine. In all cases, destruction is >99% and good accountances are obtained for the products (Table 1).

Destruction Procedures

Destruction of N*-Methylurethane, Methyl Carbamate, and Urethane*

Add 50 mg of the compound to 10 mL of 5 M NaOH solution and stir at room temperature for 24 h (MC, UT) or 48 h (MUT). Check the reaction mixture for completeness of destruction, neutralize, and discard it.

Note: This procedure is **not** suitable for EUT.

Destruction of N-Methylurethane, N-Ethylurethane, Methyl Carbamate, and Urethane

Add 50 mg of the compound to 10 mL of 5 M NaOH solution and reflux for 4 h. Cool, check the reaction mixture for completeness of destruction, neutralize, and discard it.

Analytical Procedures

The carbamic acid esters were determined by GC using a 1.8-m × 2-mm i.d. packed column filled with 5% Carbowax 20 M on 80/100 Chromosorb W HP.[7] The column was fitted with a precolumn, and it was found helpful to change it regularly. The oven temperature was 120 °C (MUT and EUT) or 140 °C (MC and UT), the injection temperature was 200 °C, and the flame ionization detector operated at 300 °C. Injection of reaction mixtures containing 5 M NaOH solution onto the hot GC column degraded any residual carbamate and gave unreliable results. To get around this, 2 mL of the

reaction mixture were acidified, before analysis, with 1 mL of concentrated hydrochloric acid (**Caution!** Exothermic!), and this mixture was then neutralized by adding solid sodium bicarbonate. Injection of this solution onto the column gave reliable results. The absence of any carbamate could be confirmed by spiking the reaction mixture with a small amount of a dilute solution of the carbamate in question. The products of the reaction were determined using the same conditions using a column packed with 10% Carbowax 20 M + 2% KOH in 80/100 Chromosorb W AW with an oven temperature of 150 °C.

The GC conditions given above are only a guide; the exact conditions would have to be determined experimentally. Using 5 µL injections, our detection limits were about 30 µg/mL (MC), 7 µg/mL (UT), and 4 µg/mL (MUT and EUT).

A method involving derivatization has been described by Wang et al.[8] Mix 500 µL of a sample in 15% ethanol, 500 µL anhydrous ethanol, 400 µL 20 mM xanthydrol in *n*-propanol, and 100 µL 1.5 M HCl. Shake for 30-sec, and allow to stand in the dark for 70–90 min. Filter (0.25 µm) and inject an aliquot. The column was a 250 × 4.6 5 µm Atlantis C18 maintained at 30 °C, the flow rate was 0.8 mL/min, and the fluorescence detector operated with excitation at 234 nm and emission at 600 nm. The mobile phase was a gradient of MeCN:20 mM sodium acetate from 45:55 to 50:50 over 10 min, to 100:0 over 25 min, maintain at 100:0 for 5 min, return to initial conditions over 1 min, and re-equilibrate for 6 min. The urea derivative eluted at about 8.3 min, and the ethyl carbamate derivative eluted at about 21.4 min. For ethyl carbamate, the limit of detection was 7 µg/L and the limit of quantitation was 20.7 µg/L.

An HPLC-MS method has also been published.[9]

Mutagenicity Assays

The mutagenicity assays were carried out as described on page 4 using tester strains TA98, TA100, TA1530, and TA1535. The final reaction mixtures (tested at a level corresponding to 0.5 mg of undegraded material per plate) were not mutagenic. The only pure compound that was mutagenic was MC (tested in DMSO solution), which was mutagenic to TA98 with activation. Ammonium carbamate, which is related to a possible intermediate in the degradation reactions, was not mutagenic.

Related Compounds

The procedure should be generally applicable to the destruction of carbamic acid esters, but it should be carefully checked to ensure that the compounds are completely degraded. The resistance to hydrolysis appears to increase as the degree of substitution increases. More highly substituted carbamic esters may require prolonged refluxing.

References

1. Other names for this compound are urethylane, Bendiocarb, and NCI-C55594.
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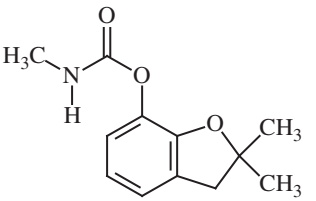
CARBOFURAN

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Carbofuran (**I**)[1] is a white crystalline solid (mp 150–153 °C) [1563-66-2], and it is a teratogen, a mutagen, and toxic by inhalation, ingestion, and skin contact.[2] This compound is used as an insecticide and it functions as a cholinesterase inhibitor. It is slightly soluble in water.



I

Principles of Destruction and Decontamination

Carbofuran in aqueous solution can be degraded using a photo-Fenton reaction (Fe^{++}/H_2O_2/UV light)[3] or by photolysis in the presence of titanium dioxide.[4] Destruction appears to be >98%, but exact figures are not quoted.

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Destruction Procedures

Caution! UV radiation is harmful to the skin and eyes. Before turning on the lamp cover the apparatus with aluminum foil or other light-absorbing material. Turn off the light before removing the covering.

Caution! Many of the lamps that are used in these procedures require a constant supply of cooling water. A device that shuts off the lamp if the flow of water fails is recommended.

Adjust the pH of a 452 μM solution of carbofuran in water to 3 with NaOH or orthophosphoric acid, add hydrogen peroxide to a final concentration of 5 mM, add ferrous sulfate to a final concentration of 500 μM, and photolyze for 10 min in an all-quartz apparatus using a Hanau TQ150 high-pressure mercury vapor lamp or equivalent with water cooling. At the end of this time, check for completeness of degradation, and discard it.[3]

Add titanium dioxide (Degussa, surface area 50 m^2/g, size 20–30 nm) at a concentration of 1.43 g/L to a solution of carbofuran in water (55 mg/L) at pH 7.60. Stir while irradiating with a xenon lamp (350 W/m^2) or solar energy for 160 min. At the end of this time, check for completeness of degradation, and discard it.[4]

Analytical Procedures

Carbofuran can be analyzed by HPLC using a Nova-Pak C18 column (Waters) and UV detection. The mobile phase was methanol:H_2O:acetic acid (45:53:2) flowing at 1 mL/min.[3] Alternatively, a Zorbax SB C18 column with UV detection at 220 nm has been recommended. The mobile phase was MeCN: water (40:60) flowing at 1 mL/min.[4]

Related Compounds

The method is specific for carbofuran, but the photo-Fenton reaction generates reactive hydroxyl radicals which can attack a wide variety of organic compounds (see the section of Procedures Classified by Method). Thorough validation would be needed for each compound.

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CHLOROMETHYLSILANES AND SILICON TETRACHLORIDE

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

The chloromethylsilanes, chlorotrimethylsilane (or trimethylsilyl chloride) [75-77-4] [$(CH_3)_3SiCl$],[1] dichlorodimethylsilane [$(CH_3)_2SiCl_2$] [75-78-5],[2] and methyltrichlorosilane (CH_3SiCl_3) [75-79-6][3] are flammable, volatile, toxic, corrosive liquids used in organic chemistry. Silicon tetrachloride ($SiCl_4$) [10026-04-7][4] is a volatile, toxic, corrosive liquid[5] used in the preparation of pure silicon, for producing smokescreens, and in inorganic chemistry. Silicon tetrachloride has been reported to react with rubber septa used to seal bottles containing this compound.[6] This causes deterioration of the septum and an overpressure inside the bottle leading to a hazardous situation. It is likely that the other chloromethylsilanes will exhibit similar behavior.

Destruction Procedure[7, 8]

Hydrolyze by cautiously adding 5 mL of the compound to 100 mL of vigorously stirred H_2O in a flask. The reaction produces hydrochloric acid and polymeric silicon-containing material. Remove any insoluble material and discard it with the solid or liquid waste. Neutralize the aqueous layer and discard it.

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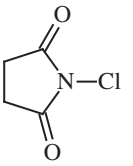
N-CHLOROSUCCINIMIDE AND CHLORAMINE-T

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

N-Chlorosuccinimide (NCS, I) [128-09-6][1] is an oxidizing and a chlorinating agent. It is used in organic chemistry. It is a white crystalline solid (mp 150–151 °C). It may be a carcinogen.[2] NCS reacts violently with aliphatic alcohols, benzylamine, and hydrazine hydrate,[3] and it has been known to smolder on storage.[2, 3] Chloramine-T (II) [127-65-1] is a crystalline solid (mp 167–170 °C).[4, 5] Chloramine-T is corrosive and liberates a toxic gas with acid.[6] Chloramine-T is supplied as the hydrate which appears to be quite stable. The anhydrous form has, however, been reported to decompose violently.[7]



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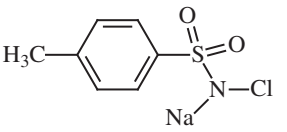

II

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Destruction Procedure[8]

Add 5 g of the compound to 100 mL of 10% (w/v) sodium metabisulfite solution and stir the mixture at room temperature. Test for completeness of destruction by adding a few drops of the reaction mixture to an equal volume of 10% (w/v) potassium iodide solution, acidifying with 1 M hydrochloric acid, and adding a drop of starch as an indicator. A deep blue color indicates the presence of excess oxidant. If destruction is complete, discard the mixture. If destruction is not complete, add more sodium metabisulfite solution until a negative test is obtained.

Related Compounds

This technique should be generally applicable to other *N*-chloro compounds.

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CHLOROSULFONIC ACID

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Chlorosulfonic acid ($ClSO_2OH$) [7790-94-5][1] is a volatile (bp 151–152 °C), corrosive liquid that is used in organic chemistry as a chlorosulfonating and condensing agent. It is used for preparing sulfate esters, sulfones, and saccharin. It reacts violently with H_2O but can be hydrolyzed in a controlled fashion by adding it to crushed ice. It is corrosive, causes severe acid burns, and it is very irritating to the eyes, lungs, and mucous membranes.[2] It reacts violently with a wide variety of compounds.[2, 3]

Destruction Procedure[4]

Cautiously add 5 mL of chlorosulfonic acid to 100 g of crushed ice. Stir the reaction mixture until it reaches room temperature and the reaction is over, neutralize, and discard it.

Related Compounds

This procedure is specific for chlorosulfonic acid. Acid halides, sulfonyl halides, and acid anhydrides can generally be degraded by adding them to 2.5 M sodium hydroxide solution (see the monograph on Acid Halides and Anhydrides).

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CHROMIUM(VI)

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Chromium(VI) is an oxidizer and a carcinogen in humans and experimental animals.[1–3] It is widely used in organic synthesis and is one of the hazardous constituents of chromic acid. Because compounds containing chromium(VI) are powerful oxidizers, they can react violently with a variety of organic and inorganic compounds.[4] Chromic acid and its salts are poisonous and corrosive to the skin and mucous membranes forming ulcers that are slow to heal.[4] Less-hazardous alternatives to chromic acid are available, and they should be used whenever possible. EOSULF, an EDTA-organosulfonate-based detergent, has been tested and found to be just as effective as chromic acid for cleaning glassware.[5]

The compounds for which this procedure has been validated are chromium trioxide [1333-82-0] [CrO_3, chromium(VI) oxide, chromic anhydride], sodium dichromate [10588-01-9] ($Na_2Cr_2O_7.2H_2O$, sodium bichromate), potassium dichromate [7778-50-9] ($K_2Cr_2O_7$, potassium bichromate), ammonium dichromate [7789-09-5] [$(NH_4)_2Cr_2O_7$, ammonium bichromate], chromic acid [a solution of chromium(VI) in concentrated sulfuric acid (H_2SO_4)], and the commercially available solution Chromerge, both in the concentrated and diluted forms.

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Principles of Destruction

Chromium(VI) is reduced to chromium(III), which is not an oxidizer, using sodium metabisulfite and the chromium(III) is precipitated by basification with magnesium hydroxide [$Mg(OH)_2$]. The IARC has reported that there is inadequate evidence that chromium(III) is carcinogenic.[3] However, chromium(III) should not be discharged to the environment[6] because it may become reoxidized to chromium(VI) in soil[7–10] or in water treatment plants.[6] Sodium or potassium hydroxide gives a gelatinous precipitate, which is hard to filter. Precipitates that are easier to filter can be obtained by careful control of the pH,[11] but $Mg(OH)_2$ automatically produces the right pH, and the sludgelike precipitate is relatively easy to filter.[12] The clear filtrate is just slightly basic (pH 7.1–9.2) and contains no trace of chromium(VI) (<0.25 ppm) and only trace amounts of chromium(III).

Destruction Procedures

Disposal of Bulk Quantities of Chromium(VI)-Containing Compounds (Sodium Dichromate, Potassium Dichromate, Ammonium Dichromate, Chromium Trioxide, and Chromerge Concentrate)

Stir the chromium compound (5 g) in 100 mL of 0.5 M H_2SO_4. When it has completely dissolved, add 10 g of sodium metabisulfite. Stir the mixture for 1 h and allow it to cool, then check for the presence of chromium(VI). Mix a few drops of the reaction mixture with a few drops of 100 mg/mL potassium iodide (KI) solution. A dark color indicates that chromium(VI) is still present. (If necessary, the color can be made more apparent by adding a drop of starch solution.) If chromium(VI) is still present, add sodium metabisulfite until a negative test is obtained. Add $Mg(OH)_2$ (6 g) to the reaction mixture and stir the mixture for 1 h, then allow it to stand overnight. Decant the mixture into a suction filter apparatus so that the clear liquid is filtered first, then the green precipitate is sucked dry. If the filtrate is yellow, this may indicate the presence of chromium(VI). Check using the KI test described above (acidify first with a little dilute H_2SO_4). If chromium(VI) is present in the filtrate, acidify with H_2SO_4 and repeat the process. The filtrate contains no trace of chromium(VI) and only traces of chromium(III). The sludge does not contain chromium(VI) but does contain large amounts of chromium(III). The sludge is, however, no longer an oxidizer. Dispose of appropriately.

If the reaction is performed on a larger scale than that described above, considerable heat is generated, particularly when the sodium metabisulfite is added, and it may be necessary to let a longer time elapse between stages to allow for cooling. The authors have performed this procedure with 550 g of potassium dichromate.

Disposal of Solutions Containing Chromium(VI) (e.g., New or Used Chromic Acid or Chromerge Solutions)

Carefully add the chromium solution (10 mL), with stirring, to 60 mL of H_2O and stir the mixture for at least 1 h until it is cool. Add sodium metabisulfite solution

(100 mg/mL, 10 mL) and stir the mixture for a few minutes, then check for the presence of chromium(VI). Mix a few drops of the reaction mixture with a few drops of 100 mg/mL KI solution. A dark color indicates that chromium(VI) is still present. (If necessary, the color can be made more apparent by adding a drop of starch solution.) If chromium(VI) is still present, add sodium metabisulfite until a negative test is obtained. Add $Mg(OH)_2$ (12 g) to the reaction mixture and stir the mixture for 1 h, then allow it to stand overnight. Decant the mixture into a suction filter apparatus so that the clear liquid is filtered first, then the green precipitate is sucked dry. If the filtrate is yellow, this may indicate the presence of chromium(VI). Check using the KI test described above (acidify first with a little dilute H_2SO_4). If chromium(VI) is present, acidify the filtrate with H_2SO_4 and repeat the process. The filtrate contains no trace of chromium(VI) and only traces of chromium(III). The sludge does not contain chromium(VI) but does contain large amounts of chromium(III). The sludge is, however, no longer an oxidizer. Dispose of appropriately.

If the reaction is performed on a larger scale than that described above, considerable heat is generated, particularly when the solution is initially diluted and when the $Mg(OH)_2$ is added, and it may be necessary to do these procedures more slowly and let a longer time elapse between stages to allow for cooling. The authors have performed this procedure with 2.2 L of new and used chromic acid.

Analytical Procedures

Total chromium can be determined by flame atomic absorption spectroscopy using a hollow cathode lamp at 357.9 nm.

A chelating pretreatment with 2,6-pyridinecarboxylic acid can be used with liquid chromatography inductively coupled plasma mass spectrometry to determine both Cr(VI) and Cr(III) in solution.[13]

Chromium(VI) may be determined using a colorimetric procedure.[14] Dissolve *sym*-diphenylcarbazide (0.20 g) in 100 mL of ethanol to prepare a reagent solution. Add 200 µL of 3 M H_2SO_4 to 3 mL of the reaction mixture and check to make sure that this mixture is acidic. Add 100 µL of the reagent solution and shake the mixture for a few seconds. Let it stand for 10 min, then determine the violet color at 540 nm against a suitable blank. About 0.25 ppm chromium(VI) produces a just visible violet color; high concentrations of chromium(VI) produce a very intense violet color that fades rapidly. If high concentrations of chromium(VI) are present, dilute the sample mixture and repeat the procedure. The response is linear to 4 ppm. The method is quite insensitive to chromium(III), but the method can be adapted to measure total chromium by oxidizing all the chromium present to chromium(VI).[14]

Mutagenicity Assays

To test the filtrates, mutagenicity assays were carried out as described on page 4 using tester strains TA98, TA100, TA1530, and TA1535. To each plate, 100 µL of filtrate was applied. No mutagenic activity was found.

Related Compounds and Related Procedures

All procedures described here are specific for chromium(VI) and should not be used for any other heavy metal. However, see also the monographs on Mercury and Heavy Metals. The procedures described above were specifically developed for dealing with chromium in the laboratory. However, a number of procedures developed for large-scale work have been described in the literature, and these procedures may have some application in the laboratory.

- Chromium(III) can be removed from the solution using IRN77 cation-exchange resin (Rohm & Haas)[15, 16] and SKN1 cation-exchange resin (Mitsubishi).[16] In a typical experiment, 100 mL of water at pH 3.5 containing 100 μg/mL Cr(III) is mostly decontaminated after shaking with 300 mg resin for 2.5 h. However, the exact level of remaining Cr(III) is not reported.
- Cr(III) can be removed from water with Amberlite IRC86 (Rohm & Haas)[17] and Amberlite IRC-50 (Na) (Rohm & Haas).[18]
- Cr(VI) can be removed using the anion-exchange resins D301, D314, and D354 (Guangzhou Mingjun Chemical Co., China).[19]
- CR(VI), as the diphenylcarbazide complex, can be removed from 25 mM sulfuric acid using Amberlite XAD-4 resin.[20]
- Shaking a suspension of 6.25 g dried and hydrated coconut coir with 100 mL of a solution containing 200 mg/L Cr(VI) at pH 2 at 200 rpm for 30 min resulted in >95% of the Cr(VI) being adsorbed on the coconut coir and removed from the solution.[21]
- Cr(VI) can be reduced to Cr(III) using scrap iron.[22] The Cr(III) is subsequently precipitated at pH 7.6–8.0.
- Cr(VI) may be removed from water using zero-valent iron.[23, 24]

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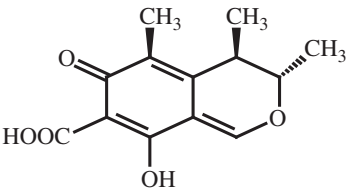
CITRININ

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures

Citrinin (**I**) [518-75-2][1] is a fungal metabolite from *Aspergillus niveus* and also from *Penicillium citrinum*. This compound is a teratogen and a severe skin irritant.[2] Citrinin may be carcinogenic in experimental animals,[3] but it has not been found to be mutagenic.[4–6] In a collaborative study organized by the International Agency for Research on Cancer (IARC), the safe disposal of citrinin was investigated.[7]



I

Citrinin is a yellow crystalline solid (mp 170–173 °C). This compound is practically insoluble in H_2O, but soluble in organic solvents. The solid compound may become electrostatically charged and cling to glassware or protective clothing.

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Principle of Destruction

Citrinin may be degraded using dilute sodium hypochlorite (NaOCl) solution and using potassium permanganate in sodium hydroxide solution ($KMnO_4$ in NaOH). Degradation efficiency was >99.5% using NaOCl and >99.9% using $KMnO_4$.[7] It has also been reported that the toxic effect of citrinin on HeLa cells can be removed by reaction with 0.05% hydrogen peroxide in an aqueous solution at room temperature for 30 min.[8] However, chemical degradation and lack of mutagenicity were not demonstrated.

Note: Sodium hypochlorite has been shown to degrade citrinin, but given the propensity of sodium hypochlorite to react to form toxic by-products (see the chapter on the use of sodium hypochlorite near the beginning of this book), it may not be advisable to use this procedure. Potassium permanganate, as described below, might be more appropriate.

Destruction Procedures[7]

Destruction of Bulk Quantities of Citrinin

1. Prepare a dilute solution of NaOCl by adding 100 mL of commercial 5.25% NaOCl solution (Clorox bleach) to 200 mL of H_2O. Use fresh NaOCl solution (see below for the assay procedure). Dissolve each 1 mg of citrinin in 2 mL of methanol. For each 2 mL of solution, add 50 mL of the dilute NaOCl solution. Sonicate to improve solubilization, allow it to react for at least 30 min, check for completeness of destruction, and discard the reaction mixture.
2. Prepare a 0.3 *M* solution of $KMnO_4$ in 2 *M* NaOH solution by stirring the mixture for at least 30 min but not more than 2 h. Dissolve 2 mg of citrinin in 5 mL of acetonitrile, and add 10 mL of $KMnO_4$ in NaOH. Stir for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of H_2O, filter to remove the manganese salts,[9] check for completeness of destruction, and discard the solid and filtrate appropriately.

Destruction of Citrinin in Aqueous Solution

1. Prepare a dilute solution of NaOCl by adding 100 mL of commercial 5.25% NaOCl solution (Clorox bleach) to 200 mL of H_2O. Use fresh NaOCl solution (see below for assay procedure). If necessary adjust the pH of the citrinin solution to neutral or alkaline. For each 1 mg of citrinin present, add 2 mL of methanol. For each 1 mg of citrinin, add 50 mL of the dilute NaOCl solution. Sonicate to improve solubilization, allow it to react for at least 30 min, check for completeness of destruction, and discard the reaction mixture.
2. Dilute with H_2O, if necessary, so that the concentration of citrinin does not exceed 200 μg/mL. Add sufficient NaOH, with stirring, to make the concentration 2 *M*,

then add sufficient solid $KMnO_4$ to make the concentration 0.3 *M*. Stir for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of H_2O, filter to remove the manganese salts,[9] check for completeness of destruction, and discard the solid and filtrate appropriately.

Destruction of Citrinin in Volatile Organic Solvents

1. Prepare a dilute solution of NaOCl by adding 100 mL of commercial 5.25% NaOCl solution (Clorox bleach) to 200 mL of H_2O. Use fresh NaOCl solution (see below for the assay procedure). Remove the solvent under reduced pressure using a rotary evaporator. Add enough methanol to wet the glass, adding at least 2 mL of methanol for each 1 mg of citrinin present. For each 2 mL of solution, add 50 mL of the dilute NaOCl solution. Sonicate to improve solubilization, allow it to react for at least 30 min, check for completeness of destruction, and discard the reaction mixture.
2. Prepare a 0.3 *M* solution of $KMnO_4$ in 2 *M* NaOH solution by stirring the mixture for at least 30 min but not more than 2 h. Remove the organic solvent under reduced pressure using a rotary evaporator. For each 2 mg of citrinin present, add 5 mL of acetonitrile, and swirl until it is dissolved. Next, add 10 mL of the solution of $KMnO_4$ in NaOH. Stir for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of H_2O, filter to remove the manganese salts,[9] check for completeness of destruction, and discard the solid and filtrate appropriately.

Destruction of Citrinin in Dimethyl Sulfoxide or N,N-Dimethylformamide

Prepare a dilute solution of NaOCl by adding 100 mL of commercial 5.25% NaOCl solution (Clorox bleach) to 200 mL of H_2O. Use fresh NaOCl solution (see below for the assay procedure). Dilute the dimethyl sulfoxide (DMSO) or *N,N*-dimethylformamide (DMF) solution with two volumes of H_2O and extract three times with equal volumes of dichloromethane, pool the extracts, and dry them over anhydrous sodium sulfate. Remove the sodium sulfate by filtration and wash it with one volume of dichloromethane. Evaporate to dryness and make sure that all the dichloromethane is removed under reduced pressure using a rotary evaporator. Add enough methanol to wet the glass, adding at least 2 mL of methanol for each 1 mg of citrinin present. For each 2 mL of the solution, add 50 mL of the dilute NaOCl solution. Sonicate to improve solubilization, allow it to react for at least 30 min, check for completeness of destruction, and discard the reaction mixture.

Decontamination of Glassware

1. Prepare a dilute solution of NaOCl by adding 100 mL of commercial 5.25% NaOCl solution (Clorox bleach) to 200 mL of H_2O. Use fresh NaOCl solution (see below for the assay procedure). Add enough methanol to wet the glassware, and immerse it in the dilute NaOCl solution for at least 30 min, check for completeness of destruction, and discard the decontaminating solution.
2. Prepare a 0.3 *M* solution of $KMnO_4$ in 2 *M* NaOH solution by stirring the mixture for at least 30 min but not more than 2 h. Rinse the glassware five times with small portions of dichloromethane. Combine the rinses and evaporate the dichloromethane under reduced pressure using a rotary evaporator. Dissolve 2 mg of citrinin in 5 mL of acetonitrile, and add 10 mL of $KMnO_4$ in NaOH. Stir for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of H_2O, filter to remove the manganese salts,[9] check for completeness of destruction, and discard the solid and filtrate appropriately.

Decontamination of Protective Clothing

Prepare a dilute solution of NaOCl by adding 100 mL of commercial 5.25% NaOCl solution (Clorox bleach) to 200 mL of H_2O. Use fresh NaOCl solution (see below for assay procedure). Add enough methanol to wet the protective clothing and immerse it in the dilute NaOCl solution for at least 30 min, check for completeness of destruction, and discard the decontaminating solution.

Decontamination of Spills

1. Prepare a dilute solution of NaOCl by adding 100 mL of commercial 5.25% NaOCl solution (Clorox bleach) to 200 mL of H_2O. Use fresh NaOCl solution (see below for the assay procedure). Collect spills of liquid with a dry cloth and spills of solid with a tissue wetted with sodium bicarbonate solution (5% w/v). Wipe the area with a cloth wetted with sodium bicarbonate solution (5% w/v). Immerse all cloths in the dilute NaOCl solution, allow it to react for at least 30 min, check for completeness of decontamination, and discard the decontaminating solution. Cover the spill area with the dilute NaOCl solution. After at least 30 min, absorb the liquid with cloths, and discard it. Check the surface for completeness of decontamination using a wipe moistened with methanol, and analyze the wipe for the presence of citrinin.
2. Prepare a 0.3 *M* solution of $KMnO_4$ in 2 *M* NaOH solution by stirring the mixture for at least 30 min but not more than 2 h. Collect spills of liquid with a dry tissue and spills of solid with a tissue wetted with dichloromethane. Immerse all tissues in the $KMnO_4$ in NaOH solution. Allow it to react for at least 3 h. The color should be either green or purple. If it is not, add more $KMnO_4$ in NaOH until the

green or purple color persists for at least 1 h. For each 10 mL of $KMnO_4$ in NaOH, add 0.8 g of sodium metabisulfite (more if necessary for complete decolorization), dilute with an equal volume of H_2O, filter to remove manganese salts,[9] check for completeness of destruction, and discard the solid and filtrate appropriately. Cover the spill area with an excess of the $KMnO_4$ in NaOH solution and allow it to react for 3 h. Collect the solution on a tissue and immerse the tissue in a 2 *M* sodium metabisulfite solution. If the pH of this solution is acidic, make it alkaline with NaOH. Rinse the spill area with a 2 *M* solution of sodium metabisulfite. Check the surface for completeness of decontamination using a wipe moistened with methanol, and analyze the wipe for the presence of citrinin.

Decontamination of Thin-Layer Chromatography Plates

Prepare a dilute solution of NaOCl by adding 100 mL of commercial 5.25% NaOCl solution (Clorox bleach) to 200 mL of H_2O. Use fresh NaOCl solution (see below for the assay procedure). Spray the plate with the dilute NaOCl solution, and allow it to react for at least 30 min. Check for completeness of destruction by scraping the plate and eluting any remaining citrinin with a suitable solvent.

Analytical Procedures

1. For reaction mixtures from the NaOCl procedures, acidify an aliquot of the final reaction mixture to pH 3–4 with concentrated hydrochloric acid (HCl) and pass nitrogen through the mixture for at least 1 min to remove chlorine. Analyze by reverse phase high-performance liquid chromatography (HPLC) using acetonitrile: 0.25 *M* aqueous phosphoric acid (75 : 25) flowing at 1.5 mL/min and a UV detector set at 254 nm or a spectrofluorimetric detector using 330 nm for excitation and 480 nm for emission.[7] An alternative mobile phase is methanol: 0.02 *M* potassium dihydrogen phosphate (pH 4.7) (50 : 50).[10] A method for determining citrinin in cereals has been described.[11] Other papers describe methods with fluorescence[12–14] and mass spectrometric[15, 16] detection.
2. For reaction mixtures obtained using the $KMnO_4$ procedures, acidify an aliquot to pH 2–3 using concentrated HCl. Extract this mixture three times with an equal volume of dichloromethane, pool the extracts, and dry them over anhydrous sodium sulfate. Remove the sodium sulfate by filtration, evaporate to dryness and take up the residue in 0.5 mL of methanol : water (75 : 25). Analyze by HPLC as above.

Mutagenicity Assays

The residues from these degradation procedures were tested using tester strains TA97, TA98, TA100, and TA102 of *Salmonella typhimurium* with and without metabolic activation. No mutagenic activity was found.[7] Citrinin has not been found to be mutagenic.[4–6]

Related Compounds

The above techniques were investigated for citrinin, but they may also be applicable to some other mycotoxins. However, these techniques should be thoroughly investigated before being applied to other compounds. See also monographs on aflatoxins, ochratoxin A, patulin, and sterigmatocystin.

Assay of Sodium Hypochlorite Solution

Sodium hypochlorite solutions tend to deteriorate with time, so these solutions should be periodically checked for the amount of active chlorine they contain. Pipette 10 mL of the NaOCl solution into a 100 mL volumetric flask and fill it to the mark with distilled H_2O. Pipette 10 mL of this solution into a conical flask containing 50 mL of distilled H_2O, 1 g of potassium iodide, and 12.5 mL of 2 *M* acetic acid. Titrate this solution against a 0.1 *N* sodium thiosulfate solution using starch as an indicator. Each 1 mL of the sodium thiosulfate solution corresponds to 3.545 mg of active chlorine. Commercially available NaOCl solution (Clorox bleach) contains 5.25% NaOCl and should contain about 45–50 g of active chlorine per liter.

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COMPLEX METAL HYDRIDES

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Complex metal hydrides are widely used in organic synthesis. These hydrides are generally air and H_2O sensitive and may be pyrophoric. Vigorous reaction with H_2O may ignite the flammable hydrocarbon solvents generally used with these reagents. In reaction with H_2O, flammable hydrogen is released.[1]

Compound Name	Reference	Formula	Registry Number
Calcium hydride	2	CaH_2	[7789-78-8]
Borane-THF complex	3	$BH_3 \cdot C_4H_8O$	[14044-65 6]
Lithium aluminum hydride	4, 5	$LiAlH_4$	[16853-85-3]
Lithium hydride	6	LiH	[7580-67-8]
Potassium hydride	7	KH	[7693-26-7]
Sodium borohydride (sodium tetrahydroborate)	8	$NaBH_4$	[16940-66-2]
Sodium cyanoborohydride (sodium cyanotrihydridoborate)		$NaBH_3CN$	[25895-60-7]
Sodium hydride	9	NaH	[7646-69-7]

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All of these reagents can react violently with a variety of organic and inorganic compounds. Sodium borohydride is toxic by ingestion,[8] borane-THF complex may explode on storage,[3] lithium hydride[6] is irritating to the skin and eyes, and sodium hydride may explode when mixed with DMSO, DMF, or *N,N*-dimethylacetamide.[10] All these compounds should be handled as if they were strong irritants and toxic. Lithium aluminum hydride may ignite when ground.[4] Excess lithium aluminum hydride may be destroyed using ethyl acetate, preferably diluted with an inert solvent.[11] As an added hazard, sodium cyanoborohydride generates one equivalent of cyanide when it is used as a reducing agent.[12]

Principles of Destruction

In general, the hydride is allowed to react slowly with H_2O, alcohol, or ethyl acetate under controlled conditions. Although sodium and potassium hydride react readily with butanol, lithium hydride does not, so it is necessary to use H_2O to destroy this compound. Sodium borohydride is more stable, and it is necessary to use acetic acid to cause decomposition. Because sodium cyanoborohydride generates an equivalent of cyanide as it reacts,[12] sodium hypochlorite (NaOCl) solution (Clorox bleach) is used. The hydride is oxidized to hydrogen chloride, and the cyanide is oxidized to the much less toxic cyanate. A typical reduction procedure with $NaBH_3CN$ involves the use of methanol as a reaction solvent.[13] The reaction takes place at pH 3 and some cyanide is evolved as the reaction proceeds. The reaction should be done in a hood but the cyanide can be removed from the exhaust gas by bubbling it through NaOCl solution. The methanol is evaporated using a rotary evaporator, and the residue is taken up in H_2O and extracted with diethyl ether. The distillate collected in the evaporator also contains cyanide. All of these solutions can be decontaminated with NaOCl solution.[14] Destruction is >99.7%. Methanol is added to solubilize the ether layer. The researcher should be aware that when the reaction mixture is evaporated most of the cyanide disappears, presumably through the exhaust of the aspirator and down the sink. This condition may be important if the reaction is carried out on a large scale.

Note: It has become increasingly apparent that the use of sodium hypochlorite leads, in many cases, to the generation of toxic products. See the monograph on sodium hypochlorite near the beginning of this book. We recommend that a risk-benefit analysis be carried out before continuing to employ sodium hypochlorite in the degradation of sodium cyanoborohydride.

Destruction Procedures

Lithium Aluminum Hydride

1. Stir the $LiAlH_4$ in a suitable solvent, and for each *n*-grams of $LiAlH_4$ present, slowly add *n* mL of H_2O under nitrogen.[15] Use an ice bath and an efficient bubbler that can deal with the large quantities of gas produced in the course of the reaction. Add *n* mL of 15% sodium hydroxide solution and 3*n* mL of H_2O in succession and

stir the mixture vigorously for 20 min. Filter the granular precipitate that forms. Separate the organic and aqueous layers of the filtrate, and discard them.

2. Stir the $LiAlH_4$ under nitrogen in a suitable solvent using an ice bath, and slowly add 7 mL of 95% ethanol for each gram of $LiAlH_4$. The 95% ethanol reacts less vigorously than H_2O.[16] Use an efficient bubbler that can deal with the large quantities of gas produced in the course of the reaction. When the reaction is complete, cautiously add a volume of H_2O equal to the initial reaction volume, separate the organic and aqueous layers, and discard them.
3. Stir the $LiAlH_4$ under nitrogen in a suitable solvent using an ice bath, and slowly add 11 mL of ethyl acetate for each gram of $LiAlH_4$. Ethyl acetate reacts less vigorously than H_2O and generates no hydrogen.[17] When the reaction is complete, add a volume of H_2O equal to the initial reaction volume, separate the organic and aqueous layers of the filtrate, and discard them.

Sodium Borohydride

Sodium borohydride is relatively stable in H_2O and acid is needed for its decomposition.[16] Dissolve the solid compound in H_2O and dilute aqueous solutions with H_2O, if necessary, so that the concentration does not exceed 3%. For each 100 mL of the solution, add 1 mL of 10% (v/v) aqueous acetic acid with stirring under nitrogen.[14] Discard when the reaction has ceased.

Borane·THF Complex

Add 30 mL of a 1 *M* solution of borane-tetrahydrofuran (BH_3-THF) in THF to 30 mL of acetone with stirring under nitrogen. After 5 min, add 30 mL of H_2O. When the reaction has ceased add 30 mL of H_2O, and discard the mixture.[14]

Sodium Hydride and Potassium Hydride

Sodium hydride and potassium hydride are generally supplied as dispersions in mineral oil. For each gram of the hydride dispersion, add 25 mL of dry isooctane (2,2,4-trimethylpentane) and stir the mixture under nitrogen. For each gram of hydride dispersion, slowly add 10 mL of *n*-butyl alcohol. Ensure that no unreacted material remains on the side of the flask. After 30 min, or when the reaction appears to have stopped, add 25 mL of cold H_2O for each gram of hydride dispersion.[14] Separate the layers, and discard them.

Lithium Hydride

Add 1 g of LiH to 50 mL of H_2O with stirring. When the reaction has finished discard the reaction mixture.[14]

Calcium Hydride

1. Add 1 g of CaH_2 to 25 mL of 95% ethanol, with stirring, under nitrogen. When the reaction has finished, add an equal volume of H_2O, and discard it.[14]
2. Add 1 g of CaH_2 to 50 g of crushed ice behind a safety shield. When the reaction has ceased, discard the reaction mixture.[14]

Sodium Cyanoborohydride

1. Dissolve each gram of solid $NaBH_3CN$ in 10 mL of H_2O.[14] If necessary, dilute reaction mixtures with H_2O, so that the concentration of $NaBH_3CN$ does not exceed 10%. Stir the $NaBH_3CN$ solution, and cautiously add 200 mL of a 5.25% NaOCl solution (Clorox bleach) for each gram of $NaBH_3CN$ present. Stir the reaction mixture for 3 h, check that the solution is still oxidizing, analyze for the complete destruction of cyanide, and discard it. Use fresh NaOCl solution (see below for the assay procedure).
2. For each 80 mL of ether extract that may contain cyanide, add 200 mL of a 5.25% NaOCl solution (Clorox bleach) and 150 mL of methanol to produce one phase. Stir the reaction mixture for 3 h, check that the solution is still oxidizing, analyze for the complete destruction of cyanide, and discard it. Use fresh NaOCl solution (see below for the assay procedure).
3. Pass the exhaust gases through 5.25% NaOCl solution (Clorox bleach). After standing for several hours, check that the solution is still oxidizing, test for completeness of destruction, and discard it. Use fresh NaOCl solution (see below for the assay procedure).

Assay of Sodium Hypochlorite Solution

Sodium hypochlorite solutions tend to deteriorate with time, so they should be periodically checked for the amount of active chlorine they contain. Pipette 10 mL of NaOCl solution into a 100 mL volumetric flask and fill to the mark with distilled H_2O. Pipette 10 mL of this solution into a conical flask containing 50 mL of distilled H_2O, 1 g of potassium iodide (KI), and 12.5 mL of 2 *M* acetic acid. Titrate this solution against 0.1 *N* sodium thiosulfate solution using starch as an indicator. Each 1 mL of the sodium thiosulfate solution corresponds to 3.545 mg of active chlorine. The NaOCl solution used in these degradation reactions should contain 45–50 g of active chlorine per liter.

Analytical Procedures

For oxidizing power. Add a few drops of the reaction mixture to an equal volume of 10% (w/v) KI solution, then acidify with a drop of 1 *M* hydrochloric acid (HCl), and add a drop of starch solution as an indicator. The deep blue color of the starch–iodine complex indicates that excess oxidant is present.

For residual cyanide.[18] Prepare the following solutions. The buffer solution is prepared by dissolving 13.6 g of potassium phosphate, monobasic (KH_2PO_4), 0.28 g of sodium phosphate, dibasic (Na_2HPO_4), and 3.0 g of potassium bromide (KBr) in distilled H_2O and making up to 1 L with distilled H_2O. Note that the presence of KBr is necessary for the assay procedure to work correctly. The reagent is prepared by stirring 3.0 g of barbituric acid in 10 mL of H_2O and adding 15 mL of 4-methylpyridine, and 3 mL of concentrated HCl while continuing to stir. After cooling the solution is diluted to 50 mL with H_2O. The sodium ascorbate solution is 10 mg/mL in H_2O, the NaCN solution is 100 mg/L in H_2O, and the chloramine-T solution is 10 mg/mL in H_2O (*not* 100 mg/mL as stated in the paper[18]). The sodium ascorbate and chloramine-T solutions are prepared fresh daily and the standard NaCN solution is prepared fresh every week. The other solutions appear to be quite stable.

Centrifuge two portions of the reaction mixture, if necessary to remove suspended solids, and add 1 mL of each to 4 mL of buffer. If an orange or yellow color appears, add sodium ascorbate solution dropwise until the mixtures are colorless (but do not add more than 2 mL). Spike one solution with 200 µL of NaCN solution, and add 1 mL of Chloramine-T solution to each solution. Shake the solutions and allow them to stand for 1–2 min, then add 1 mL of the reagent and shake the mixtures and allow them to stand for 5 min. Blue color indicates the presence of cyanide. For complete destruction, the unspiked solution should be colorless and the spiked solution should be blue. Measure the absorbance at 605 nm using 10-mm cuvettes (after centrifuging again if necessary to remove suspended solids). Appropriate standards and blanks should always be run. The limit of detection is about 3 µg/mL.

Related Compounds

Complex metal hydrides vary greatly in their reactivity and the application of any of the above methods to another hydride should be carefully, and cautiously, investigated before employing it. In general, one should start with a less reactive substrate, such as butanol, and then, if necessary, move on to more reactive substrates, such as 95% ethanol and H_2O.

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CYANIDES AND CYANOGEN BROMIDE

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Inorganic cyanides are acutely toxic compounds, for example, the LD_{50} in the rat is 15 mg/kg for sodium cyanide (NaCN) [143-33-9] [1] and 10 mg/kg for potassium cyanide (KCN) [151-50-8].[2] These compounds should be handled with great care and not allowed to come in contact with acid that will generate hydrogen cyanide (HCN) [74-90-8], a volatile, highly toxic, flammable gas,[3] which forms explosive mixtures with air. Acute cyanide poisoning is rapidly fatal. Less acute cases of cyanide poisoning may produce symptoms, such as headache, dizziness, nausea, and so on.[3, 4] Inorganic cyanides have a number of uses in organic synthesis. Cyanogen bromide (other names bromine cyanide and CNBr) [506-68-3] is a highly toxic, volatile crystalline solid (mp 49–51 °C) with its toxic effects being those of HCN.[5] A concentration of 92 ppm for 10 min has caused a fatality[6] and low concentrations (10 ppm) are irritating to the eyes, nose, and respiratory tract.[5, 6] It should be handled with great care only in a properly functioning chemical fume hood. Cyanogen bromide is used in laboratories for the activation of agarose beads in affinity chromatography,[7] in the analysis of protein structure,[8] and for the detection of pyridine compounds.[9]

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Principles of Destruction

Inorganic cyanides and CNBr are oxidized by sodium or calcium hypochlorite [NaOCl or $Ca(OCl)_2$],[10, 11] in basic solution, to the much less toxic cyanate ion (mouse LD_{50} for sodium cyanate = 260 mg/kg[12]). Further hydrolysis of the cyanate ion is also possible. Some reactions (e.g., reduction with sodium cyanoborohydride) release HCN. This compound can be removed by passing the exhaust gases through the NaOCl solution. Cyanide can also be oxidized to bicarbonate and ammonia by hydrogen peroxide in the presence of a copper catalyst.[13] Using UV irradiation enables the use of a lesser amount of hydrogen peroxide and produces bicarbonate and nitrogen. In each case, destruction is >99.9%.[13] Photocatalytic methods using UV-LEDs, and titanium dioxide[14] or copper[15] catalysts have been reported. Destruction efficiency is 99–100%.

Note: Previous editions of this book have described methods using sodium hypochlorite for the degradation of various compounds. However, it has become increasingly apparent that the use of sodium hypochlorite leads, in many cases, to the generation of toxic products. See the monograph on sodium hypochlorite near the beginning of this book. We recommend that a risk-benefit analysis be carried out before continuing to employ sodium hypochlorite for this degradation procedure.

Destruction Procedures

Destruction of Bulk Quantities[10]

1. Dissolve NaCN or CNBr in H_2O, so that the concentration does not exceed 25 mg/mL for NaCN or 60 mg/mL for CNBr. Mix one volume of this solution with one volume of sodium hydroxide (NaOH) solution (1 *M*) and two volumes of 5.25% NaOCl [i.e., cyanide solution: NaOH:NaOCl (1:1:2)]. Stir the mixture for 3 h, test for completeness of destruction, neutralize, and discard it. Use fresh NaOCl solution (see below for the assay procedure).
2. Dissolve NaCN or CNBr in H_2O, so that the concentration does not exceed 25 mg/mL for NaCN or 60 mg/mL for CNBr. Mix one volume of this solution with one volume of NaOH solution (1 *M*) and add 60 g of $Ca(OCl)_2$ per liter of basified solution. Stir the mixture for 3 h, test for completeness of destruction, neutralize, and discard it.

Destruction of Sodium Cyanide or Cyanogen Bromide in Solution[10]

1. If necessary dilute the NaCN solution with H_2O, so that the concentration does not exceed 25 mg/mL. If necessary dilute aqueous solutions of CNBr with H_2O, so that the concentration does not exceed 60 mg/mL. If necessary dilute solutions of CNBr in organic solvents with the same organic solvent, so that the concentrations do not exceed 60 mg/mL for acetonitrile, 30 mg/mL for dimethyl sulfoxide (DMSO), *N, N*-dimethylformamide (DMF), 2-methoxyethanol, or 0.1 *M* hydrochloric acid (HCl), 25 mg/mL for ethanol, or 19 mg/mL for *N*-methyl-2-pyrrolidinone. For each volume of the solution, add one volume of 1 *M* NaOH solution and two

volumes of 5.25% NaOCl solution. Stir the mixture for 3 h, test for completeness of destruction, neutralize, and discard it. Use fresh NaOCl solution (see below for assay procedure).

2. If necessary dilute the NaCN solution with H_2O, so that the concentration does not exceed 25 mg/mL. If necessary dilute aqueous solutions of CNBr with H_2O, so that the concentration does not exceed 60 mg/mL. If necessary dilute solutions of CNBr in organic solvents with the same organic solvent, so that the concentrations do not exceed 60 mg/mL for acetonitrile, 30 mg/mL for DMSO, DMF, 2-methoxyethanol, or 0.1 *M* HCl, 25 mg/mL for ethanol, or 19 mg/mL for *N*-methyl-2-pyrrolidinone. For each volume of the solution, add one volume of 1 *M* NaOH solution, then add 60 g of $Ca(OCl)_2$ per liter of basified solution. Stir the mixture for 3 h, test for completeness of destruction, neutralize, and discard it.

Destruction of Cyanogen Bromide in 70% Formic Acid[10]

1. If necessary dilute the solution, so that the concentration of CNBr does not exceed 60 mg/mL, and basify the solution by the **slow** addition of two volumes of 10 *M* potassium hydroxide (KOH) solution (a **very** exothermic process). Cool, then for each volume of the solution, add one volume of 1 *M* NaOH solution and two volumes of 5.25% NaOCl solution. Stir the mixture for 3 h, test for completeness of destruction, neutralize, and discard it. Use fresh NaOCl solution (see below for assay procedure).
2. If necessary dilute the solution, so that the concentration of CNBr does not exceed 60 mg/mL, and basify the solution by the **slow** addition of two volumes of 10 *M* KOH solution (a **very** exothermic process). Cool, then for each volume of the solution, add one volume of 1 *M* NaOH solution, then add 60 g of $Ca(OCl)_2$ per liter of basified solution. Stir the mixture for 3 h, test for completeness of destruction, neutralize, and discard it.

Destruction of Hydrogen Cyanide[11]

Dissolve the HCN in several volumes of ice water, and add one molar equivalent of NaOH solution at 0–10 °C. (Do **not** add NaOH, NaCN, or any base to liquid HCN; a violent reaction may occur.) Add a 50% excess of 5.25% NaOCl solution (80 mL of solution for each gram of HCN) at 0–10 °C with stirring and allow the mixture to warm to room temperature. After standing for several hours, test for completeness of destruction, neutralize, and discard it. Use fresh NaOCl solution (see below for the assay procedure).

Decontamination of Hydrogen Cyanide From Exhaust Gases[16]

Pass the exhaust gases through a 5.25% NaOCl solution. After standing for several hours, test for completeness of destruction, neutralize, and discard it. Use fresh NaOCl solution (see below for the assay procedure).

Destruction of Cyanide in Solution with Hydrogen Peroxide[13]

Caution! 30% H_2O_2 is a corrosive irritant and a strong oxidizer.

1. To an aqueous solution containing 100 mg/L cyanide at pH 7.0–10.0 add copper sulfate so as to achieve a copper concentration of 75 mg/L and add hydrogen peroxide, so as to achieve a hydrogen peroxide concentration of 88.2 m*M*, stir for at least 90 min, check for completeness of destruction, and discard it.
2. **Caution!** Ultraviolet (UV) radiation is harmful to the skin and eyes. Before turning on the lamp, cover the apparatus with aluminum foil or other light-absorbing material. Turn off the light before removing the covering. To an aqueous solution containing 100 mg/L cyanide at pH 10.0 add copper sulfate, so as to achieve a copper concentration of 19 mg/L and add hydrogen peroxide, so as to achieve hydrogen peroxide concentration of 35.3 m*M*. Pass air through the solution at 1 L/min, irradiate using a 25 W low-pressure UV lamp in a borosilicate reactor for at least 9 min, check for completeness of destruction, and discard it.

Destruction of Cyanide in Solution Using Photocatalytic Procedures

Caution! UV radiation is harmful to the skin and eyes. Before turning on the lamp, cover the apparatus with aluminum foil or other light-absorbing material. Turn off the light before removing the covering.
Caution! Many of the lamps that are used in these procedures require a constant supply of cooling water. A device that shuts off the lamp if the flow of water fails is recommended.

1. Stir 500 mL of an aqueous solution containing 0.05 g/L titanium dioxide (Degussa P25) and 30 mg/L cyanide and irradiate it with a Philips UVC low-pressure mercury lamp emitting at 180–280 nm for 5 h.[14] Pass oxygen through the solution at 1.5 mL/min. Vent any evolved HCN into a fume hood. Check for completeness of destruction, and discard it.
2. Stir 500 mL of an aqueous solution containing 0.05% copper (as copper sulfate pentahydrate), 0.05% hydrogen peroxide, and 100 mg/L cyanide at pH 11 and irradiate it with a Lichtzen LED lamp emitting at 275 nm for 1 h.[15] Check for completeness of destruction, and discard it.

Assay of Sodium Hypochlorite Solution

Sodium hypochlorite solutions tend to deteriorate with time, so they should be periodically checked for the amount of active chlorine they contain. Pipette 10 mL of NaOCl solution into a 100 mL volumetric flask and fill to the mark with distilled H_2O. Pipette 10 mL of this solution into a conical flask containing 50 mL of distilled H_2O, 1 g of potassium iodide, and 12.5 mL of 2 *M* acetic acid. Titrate this solution against 0.1 *N* sodium thiosulfate solution using starch as an indicator. Each 1 mL of the sodium

thiosulfate solution corresponds to 3.545 mg of active chlorine. The NaOCl solution used in these degradation reactions should contain 45–50 g of active chlorine per liter.

Analytical Procedures[10]

Prepare the following solutions. The buffer solution was prepared by dissolving 13.6 g of potassium phosphate, monobasic (KH_2PO_4), 0.28 g of sodium phosphate, and dibasic (Na_2HPO_4), and 3.0 g of potassium bromide (KBr) in distilled H_2O and making up to 1 L with distilled H_2O. Note that the presence of KBr is necessary for the assay procedure to work correctly. The reagent was prepared by stirring 3.0 g of barbituric acid in 10 mL of H_2O, adding 15 mL of 4-methylpyridine, and 3 mL of concentrated HCl while continuing to stir. After cooling the solution was diluted to 50 mL with H_2O. The sodium ascorbate solution was 10 mg/mL in H_2O, the NaCN solution was 100 mg/L in H_2O, and the Chloramine-T solution was 10 mg/mL in H_2O (*not* 100 mg/mL as stated in the paper[10]). The sodium ascorbate and chloramine-T solutions were prepared fresh daily and the standard NaCN solution was prepared fresh every week. The other solutions appeared to be quite stable.

Two portions of the reaction mixtures were centrifuged, if necessary to remove suspended solids, and 1 mL of each was added to 4 mL of buffer. If an orange or yellow color appeared, sodium ascorbate solution was added dropwise until the mixtures were colorless (but not more than 2 mLshould be added). One solution was spiked with 200 µL of NaCN solution, and 1 mL of chloramine-T solution was added to each solution. The solutions were shaken and allowed to stand for 1–2 min, then 1 mL of the reagent was added and the mixtures were shaken and allowed to stand for 5 min. Blue color indicates the presence of cyanide. For complete destruction, the unspiked solution should be colorless and the spiked solution should be blue. The absorbance was measured at 605 nm using 10-mm cuvettes (after centrifuging again if necessary to remove suspended solids). Appropriate standards and blanks should always be run. The limit of detection was about 3 µg/mL.

Procedures for the determination of free cyanide and cyanate using derivatization followed by spectrometric analysis have been described.[17] as has a method for the quantification of cyanide in human whole blood using ion chromatography with amperometric detection.[18]

Mutagenicity Assays

Since CNBr or cyanide ion has not been reported to be mutagenic, no studies were performed.

Related Compounds and Related Procedures

These procedures should be applicable to cyanogen chloride [506-77-4], cyanogen iodide [506-78-5], and various inorganic cyanides, although we have carried out no tests. Similar procedures can be used for the destruction of sodium cyanoborohydride

(see Complex Metal Hydrides monograph). These procedures are not applicable to organic nitriles (see the Organic Nitriles monograph for methods of disposing of these compounds). The destruction of cyanide waste solutions has been described.[19] This paper also describes other industrial methods of cyanide disposal. Cyanide can be degraded using hydrogen peroxide in the presence of copper-impregnated pumice (unfortunately not commercially available).[20]

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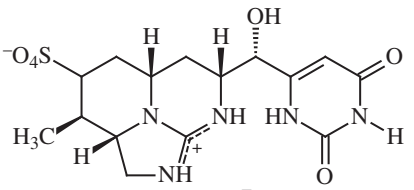
CYLINDROSPERMOPSIN

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Cylindrospermopsin[1] [**I,** 143545-90-8] is a naturally occurring tricyclic cytotoxic alkaloid produced by *Cylindrospemopsis raciborskii,* and other species within the genera of *Umezakia, Aphanizomenon, Raphidiopsis, Anabaena,* and *Lyngbya*.[2] It is a white powder soluble in water, methanol, and DMSO.[3, 4] The pure compound is relatively stable to sunlight[5, 6]; however, when a solution of the algal extract is exposed, cylindrospermopsin decomposes rapidly.[5] When heated to decomposition, it gives off NO_x and SO_x vapors.[7]



I

Oral ingestion may lead to gastroenteritis, hepatitis, renal malfunction, and hemorrhage.[8] The toxin has poisoned humans[9] and animals.[8] The LD_{50} in mice has

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been estimated as 2.1 mg/kg at 24 h and 0.2 mg/kg at 5–6 days.[10] It is a mild skin irritant.[11] There have been reports of cylindrospermopsin's activity as a mutagen and carcinogen,[12] a genotoxin,[13, 14] and a teratogen.[15]

Principle of Destruction

Cylindrospermopsin may be oxidized using ozone.[16, 17] Destruction is >95%. The products of the reaction are not known. Cylindrospermopsin can be degraded using photolysis,[18–20] it can be removed from wastewater using powdered activated carbon,[21] and it can be degraded (98%) using Oxone® in the presence of cobalt sulfate.[22]

Destruction Procedures

Caution! Ozone is an irritant! These reactions should be carried out in a properly functioning fume hood.

Caution! Ultraviolet (UV) radiation is harmful to the skin and eyes. Before turning on the lamp, cover the apparatus with aluminum foil or other light-absorbing material. Turn off the light before removing the covering.

Caution! Many of the lamps that are used in these procedures require a constant supply of cooling water. A device that shuts off the lamp if the flow of water fails is recommended.

Add ozone to a 415 µg/L solution of cylindrospermopsin in water to achieve an ozone concentration of 0.5 mg/L, test for completeness of destruction, and discard it.[16] These experiments were carried out with natural lake water having pH 8 and dissolved organic carbon of 3.6 mg/L. Other organic species may influence the efficiency of destruction.

Purge 5 mL of an aqueous solution containing 10 mg/L cylindrospermopsin and 200 mg/L Degussa P25 TiO_2 with oxygen for 20 min, then stir and irradiate with four F15 W/T8 black light tubes (Sylvania) at 365 nm for 30 min then check for completeness of destruction, and discard it.[18, 19] Destruction is "complete."

Photolyze water containing 20 µM cylindrospermopsin and 0.5 mM hydrogen peroxide with a lamp emitting at UV 254 nm for about 2 h.[20] Check for completeness of destruction, and discard it. Destruction is estimated at about 95%. Organic material in the water may slow degradation.

Allow a solution containing 10 µM cylindrospermopsin, 80 µM Oxone®, and 40 µM cobalt (II) sulfate to react at 20 °C for 15 min. The pH is not controlled and it is approximately 4.2. Quench with ethanol (160 mM), check for completeness of destruction, and discard it. The authors state that degradation of the cylindrospermopsin resulted in solutions of lower toxicity.[22]

Analytical Procedures

Cylindrospermopsin was determined by HPLC using a 55-mm × 4-mm Purospher STAR RP-18e column protected by a 4-mm × 4-mm guard column and a mobile phase of MeOH:water:trifluoroacetic acid 2:98:0.05 for 5 min–70:30:0.05 over 0.1 min,

maintained at 70:30:0.1 for 1.9 min, returned to initial conditions over 0.1 min and re-equilibrated at initial conditions for 9.9 min.[21] The flow rate was 1 mL/min and the column temperature was 40 °C. The retention time was 1.9 min, UV detection was 200–300 nm (other authors have used 262 nm[2] and 261 nm[23]), the injection volume was 25 µL, and the limit of quantitation was 49 ng/mL.

An LC–MS procedure with a limit of detection of 200 pg/mL has also been described.[24] A 150-mm × 4.6-mm Alltima C18 column was used with a gradient of MeOH:5 m*M* ammonium acetate from 2:98 to 60:40 (time not specified, but appears to be about 10 min). The flow rate was 0.8 mL/min (with 0.25 mL/min entering the detector) and the column temperature was 35 °C. The retention time was 6.5 min and a PE/Sciex API 300 detector with a turbo spray interface was used. The multiple reaction monitoring mode was used with nitrogen as the collision gas, and the transition of 416.2–194 was used for cylindrospermopsin.

Related Compounds

This procedure is specific for cylindrospermopsin.

References

1. Other names for this compound are: 6-[(*R*)-hydroxy[(2a*S*,3*R*,4*S*,5a*S*,7*R*)-2,2a,3,4,5,5a,6,7-octahydro-3-methyl-4-(sulfoxy)-1*H*-1,8,8b-triazaacenaphthalen-7-yl]methyl]-2,4(1*H*, 3*H*)-pyrinidine-dione, cylindro-spropsin, cylindrospermopsine, CYN, CYL.
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DIISOPROPYL FLUOROPHOSPHATE

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Diisopropyl fluorophosphate [55-91-4] [DFP, $[(CH_3)_2CHO]_2P(O)F$][1] is a practically odorless,[2] colorless, volatile (bp 73 °C/16 mmHg[2]) liquid that is widely used as an enzyme inhibitor.[3–9] The density is 1.055 g/mL.[10] This compound is also used to treat glaucoma.[11] Diisopropyl fluorophosphate is highly toxic (LC_{50} 360 µg/L for rats, LD_{50} 500 µg/kg for rabbits) with toxicity comparable to hydrogen cyanide.[2] This compound is a cholinesterase inhibitor,[11, 12] a neurotoxin,[13] and has reproductive effects.[14]

Principle of Destruction

Diisopropyl fluorophosphate can be degraded by adding 1 *M* sodium hydroxide (NaOH) to bulk quantities of DFP and solutions of DFP in buffer, H_2O, or *N,N*-dimethylformamide (DMF).[15] The product is diisopropylphosphate.[2, 16] The pH should be greater than or equal to 12. For solutions of DFP in buffer or H_2O, the destruction efficiency was >99.8%, for solutions of DFP in DMF the destruction efficiency was >99.97%, and when bulk quantities of DFP were degraded the destruction efficiency was >99.98%. When bulk quantities are degraded, a limitation is the speed at which the oily DFP dissolves in the NaOH. Agitation speeds up the dissolution

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Table 1 Degradation of a 1 m*M* Solution of DFP in Buffer

Buffer	pH	Time Until Addition of Buffer Solution Did Not Degrade Chymotrypsin Activity by >50% (h)
pH 3	3.0	>330
pH 5	5.0	>330
Hanks'	6.4	>330
pH 7	7.0	168
PBS	7.2	330
Dulbecco's	7.2	330
HEPES	7.5	168
TRIS	8.0	168
pH 9	9.1	18
pH 11	11.0	4

and decreases the decontamination time. Destruction was complete in 2 h, however, even when the reactants were just placed together with minimal agitation. The supplier (Aldrich Chemical Co., Milwaukee, WI) recommends the use of 2% (0.5 *M*) NaOH[17] and 1 *M* NaOH in 50% ethanol has been recommended[3] although validation details are not given. The nerve gases sarin (GB) [18–20] and soman (GD),[20] which also possess P–F bonds, are readily hydrolyzed by a strong base. The stability of DFP in buffer solution has been determined.[21] Table 1 shows the time required until the addition of buffer solution containing DFP did not degrade chymotrypsin activity by more than 50%. At this point, the amount of DFP was <0.17% of the initial amount. The stability is pH dependent and decreases markedly as the pH increases. Diisopropyl fluorophosphate is somewhat less stable in pH 7 buffer than in phosphate-buffered saline (PBS) or Dulbecco's buffer (both pH 7.2), but this may be due to the higher phosphate concentration in the pH 7 buffer (50 m*M*) than in the other buffers (10 m*M*).

Destruction Procedures[15]

Destruction of Diisopropyl Fluorophosphate in Buffer or Water

To each 1 mL of 10 m*M* DFP in buffer or H_2O, add 200 µL of 1 *M* NaOH and check that the reaction mixture is strongly basic (pH ≥ 12). Allow it to stand at room temperature for 18 h, analyze for completeness of destruction, neutralize, and discard it.

Destruction of Diisopropyl Fluorophosphate in N,N-*Dimethylformamide*

To each 1 mL of 200 m*M* DFP in DMF, add 2 mL of 1 *M* NaOH and check that the reaction mixture is strongly basic (pH ≥ 12). Allow it to stand at room temperature for 18 h, analyze for completeness of destruction, neutralize, and discard it.

Destruction of Bulk Quantities of Diisopropyl Fluorophosphate

To each 40 µL of pure DFP, add 1 mL of 1 *M* NaOH and check that the reaction mixture is strongly basic (pH ≥ 12). Stir at room temperature for 1 h, analyze for completeness of destruction, neutralize, and discard it.

Decontamination of Spills or Equipment

To decontaminate spills or equipment, add at least 1 mL of 1 *M* NaOH for each 40 µL of DFP that is estimated to be present. Add more NaOH if required to thoroughly wet all contaminated surfaces. Check that the reaction mixture is strongly basic (pH ≥ 12) and make sure that all the oily DFP has dissolved. Allow it to stand at room temperature for at least 2 h and preferably 18 h, analyze the solution for completeness of destruction, neutralize, and discard it. Decontamination is more efficient if it is possible to agitate or stir the reaction mixture (e.g., when the inside of a flask is being decontaminated). Clean the equipment or the spill area in a conventional fashion.

Buffers

The buffer solutions employed when the destruction of DFP in buffer was investigated were Hanks' Balanced Salts (Hanks') (pH 6.4), and Dulbecco's phosphate buffered saline (Dulbecco's) (pH 7.2), and PBS (pH 7.2), which were purchased from Sigma. Hanks' was 0.78 m*M* in phosphate and Dulbecco's and PBS were 10 m*M* in phosphate. The Tris-Borate-EDTA (TBE) buffer contained tris(hydroxymethyl)aminomethane (TRIS, 50 m*M*, 6.1 g/L), boric acid (5.5 m*M*, 0.34 g/L), and ethylenediaminetetraacetic acid (EDTA, 1.7 m*M*, 0.5 g/L) and was adjusted to pH 8.0 with 1 *M* hydrochloric acid (HCl). The pH 3 buffer was a 50 m*M* phthalate buffer (10.2 g of potassium hydrogen phthalate in 1 L of H_2O adjusted to pH 3.0 with 1 *M* HCl) and the pH 5 buffer was a 50 m*M* phthalate buffer (10.2 g of potassium hydrogen phthalate in 1 L of H_2O adjusted to pH 5.0 with 1 *M* NaOH). The pH 7 buffer was a 50 m*M* phosphate buffer (6.8 g of potassium dihydrogen phosphate in 1 L of H_2O adjusted to pH 7.0 with 1 *M* NaOH). The pH 9 buffer was 50 m*M* borax (19 g/L, actual pH 9.1) and the pH 11 buffer was 50 m*M* sodium carbonate (6.2 g/L). The 4-(2-hydroxyethyl)-L-piperazineethanesulfonic acid (HEPES) buffer was 50 m*M* HEPES (11.9 g/L) and 500 m*M* NaCl (29.25 g/L) adjusted to pH 7.5 with 1 *M* NaOH.

Analytical Procedures

The analytical system measures the rate at which *N*-benzoyl-L-tyrosine ethyl ester (BTEE) is hydrolyzed by chymotrypsin[15] using a procedure based on the method of Hummel[22] with the modifications of Rao and Lombardi.[23] The DFP inhibits the activity of chymotrypsin, so the rate of hydrolysis acts as an indicator of the presence or absence of DFP. The hydrolysis of BTEE is measured by determining the increase in absorbance at 256 nm. There are a number of complicating features, however, which make this analysis rather less straightforward than the average colorimetric procedure. The initial absorbance of the BTEE is quite high [typically ca. 2 AU (absorbance units)] and the increase in absorbance is small (typically 0.2–0.4 AU). Consequently, a conventional blank cannot be used because small differences in the BTEE solution or the ultraviolet (UV) cell might have large effects on the initial absorbance, and hence on the final absorbance value. Another complicating factor is that, in order to measure low concentrations of DFP, low concentrations of chymotrypsin must be used. Consequently, the reactions take a long time, typically 16–20 h. Because the analytical

system uses an enzyme, some variability was seen in the measured rates of hydrolysis and so it was necessary to run a number of controls.

The best procedure is to add a buffered aliquot of the reaction mixture to a solution of chymotrypsin, and then wait for 1 h for any DFP present to inhibit, or partially inhibit, the enzyme. The BTEE solution is then added and the absorbance is immediately determined. After standing for 16–20 h the final absorbance of the same solution in the same cell is measured and hence the rate of increase in absorbance [R_{rm}, in milliabsorbance units per hour (mAU/h)] can be calculated. At the same time, the spontaneous hydrolysis rate of BTEE is determined using a blank that contains neither enzyme nor DFP (R_{sp}). The spontaneous hydrolysis of BTEE (typically ca. 6 mAU/h) is significant over the long time course of the reaction at the pH (7.8) employed. A reaction blank that contains an enzyme, but no DFP is also run to determine the rate of hydrolysis due to both spontaneous and enzymatic hydrolysis (R_{rb}). The reaction blank was processed in exactly the same way as the degradation experiment except that no DFP was added. By subtraction the activity of the enzyme, measured by measuring the rate of enzymatic hydrolysis (typically ca. 12 mAU/h) can be calculated, $A_{rb} = R_{rb} - R_{sp}$. This value is compared with the activity of the enzyme when the reaction mixture being tested for DFP was present, $A_{rm} = R_{rm} - R_{sp}$. The lower limit of detection for DFP was set by the concentration of DFP, which would produce 50% inhibition of the activity of the enzyme, that is, $A_{rm} = 0.5A_{rb}$. Note that this does **not** represent a 50% decrease in the initial concentration of DFP because the initial concentration of DFP was many times that is needed to completely inhibit the chymotrypsin. Spiking experiments were used to determine the limit of detection.

Prepare the following solutions:

Tris Buffer. Dissolve 12.1 g of TRIS base and 14.7 g of calcium chloride dihydrate in about 900 mL of distilled H_2O. Adjust the pH to 7.8 with 1 *M* HCl and make-up to 1 L with distilled H_2O. The buffer is 100 m*M* in Tris and 100 m*M* in calcium.

MOPS Buffer. Dissolve 20.9 g of MOPS in about 900 mL of distilled H_2O. Adjust the pH to 6.5 with 1 *M* NaOH and make-up to 1 L with distilled H_2O. The buffer is 100 m*M* in MOPS.

MOPS/Ca^{2+} Buffer. Prepare as above, but add 14.7 g of calcium chloride dihydrate. The buffer is 100 m*M* in calcium.

BTEE Solution. Stir 400 µL of Triton X-100 in 200 mL of Tris buffer for about 5 min, then add a solution of 31.34 mg of BTEE in 1 mL of methanol and stir until all the BTEE dissolves. The concentration of BTEE is 0.5 m*M*. The solution should be prepared immediately before use, but is usable for several hours.

DFP Solutions. The DFP was stored in the refrigerator. It was supplied in a vial with a septum and portions were removed as required using a Hamilton 100 µL syringe. Prepare a 200 m*M* (37 mg/mL) solution by dissolving 36 µL of DFP in 1 mL of DMF. Prepare a 1 mg/mL solution by adding 100 µL of the 200 m*M* solution to 3.6 mL of DMF. These solutions should be prepared fresh each week. Prepare a 3.1 µg/mL solution of DFP in MOPS by adding 50 µL of the 1 mg/mL solution of DFP in DMF to 16 mL of MOPS (not MOPS/Ca^{2+}) buffer. This solution should be prepared and used immediately. Solutions

Table 2 Typical Set of Analytical Procedures

Analytical Procedure	Sample	Spike[a]	Enzyme or 1 m*M* HCl
Reaction mixture	Reaction mixture	MOPS	Enzyme
Spiked reaction mixture	Reaction mixture	DFP	Enzyme
Reaction blank	Reaction mixture blank	MOPS	Enzyme
Spontaneous hydrolysis blank	0.2 *M* NaOH	MOPS	HCl

[a] Spiked with MOPS buffer or 3.1 μg/mL DFP in MOPS.

of DFP in buffer and H_2O that were 10 m*M* were prepared by adding 50 μL of a 200 m*M* solution of DFP in DMF to 950 μL of buffer or H_2O.

Chymotrypsin Solutions. Prepare a 1 mg/mL solution of α-chymotrypsin (Type II from bovine pancreas, Sigma Cat. No. C 4129) using ice-cold 1 m*M* HCl. This solution should be stored in the refrigerator and prepared fresh each week. Prepare the working 0.1 μg/mL solution of chymotrypsin by diluting an aliquot of the 1 mg/mL solution 1:10,000 times with ice-cold 1 m*M* HCl. This solution should be kept on ice and prepared fresh each day.

Procedure

Perform the destruction procedure as detailed above. At the same time, prepare a reaction mixture blank as follows. Add 50 μL of DMF to 950 μL of buffer, then add 200 μL of 1 *M* NaOH. Allow this mixture to stand at room temperature for 18 h, then proceed. Add 100 μL of the reaction mixture to be tested to 1 mL of MOPS/Ca^{2+} buffer. [Buffers that contain phosphate (particularly pH 7 buffer, Dulbecco's buffer, and PBS) may produce a precipitate of calcium phosphate that will interfere with the spectrophotometric determination. If this is the case, add 0.5 mL of the reaction mixture to 5 mL of MOPS/Ca^{2+} buffer, allow it to stand for several hours, then centrifuge. Use 1.1 mL of the supernatant.] To each sample add either 100 μL of MOPS buffer or 100 μL of 3.1 μg/mL DFP in MOPS (for spiked reactions). Finally, add 100 μL of the 0.1 μg/mL chymotrypsin solution. The blank for measuring the spontaneous hydrolysis rate consists of 100 μL of 0.2 *M* NaOH, 1 mL of MOPS/Ca^{2+} buffer, 100 μL of MOPS buffer, and 100 μL of 1 m*M* HCl.

Allow these solutions to stand at room temperature for 1 h then add 3 mL of the BTEE solution, place this mixture in a quartz UV cell, and immediately determine the absorbance against an air blank. After 16–20 h again determine the absorbance and calculate the rate of hydrolysis in milli-absorbance units per hour (mAU/h). Table 2 lists a typical set of analytical procedures.

When neat DFP is degraded with NaOH solution the procedure is the same except that only 40 μL of the reaction mixture is added to 1 mL of MOPS/Ca^{2+} buffer in order to keep the pH within an acceptable range. This procedure reduced the sensitivity of the analytical technique somewhat, but the high initial concentration of DFP more than compensated for this. Sodium hydroxide (1 *M*) was used as a reaction mixture blank.

With the concentrations specified above the rate of hydrolysis (and hence, the rate of increase in absorbance) should be linear. However, this should be checked periodically by measuring the absorbance every hour and graphing the readings. The result should be a straight line. If the concentration of the enzyme is too high, the graph will rise sharply, then level off as all the BTEE is consumed. If the concentration of enzyme is too low, the graph will differ little from that of spontaneous hydrolysis.

The absorbance of the analytical samples was determined at 256 nm using quartz UV cells in a Perkin-Elmer Lambda 2 UV/Vis spectrometer fitted with a 13-cell changer. The cells were cleaned with chromic acid, which was discarded appropriately [see the monograph on Chromium(VI)]. If required, the spectrometer could be programmed to record periodically the absorbance of each cell, so that the progress of the reaction could be followed graphically. The temperature in the cell compartment was 28 °C.

Mutagenicity Assays

The mutagenicity assays were carried out as described on page 4. Tester strains TA98, TA100, TA1530, and TA1535 were used. Before testing, reaction mixtures from the destruction of DFP in buffer, H_2O, or DMF were neutralized by the addition of 100 µL of glacial acetic acid to each milliliter of the reaction mixture, and reaction mixtures obtained by the degradation of neat DFP were neutralized by the addition of 200 µL of glacial acetic acid to each milliliter of the reaction mixture. In general, the reaction mixtures were not mutagenic.[15] In some cases, mutagenic activity toward strains TA1530 and TA1535 with and without activation was seen when DFP in TBE and Dulbecco's buffers was degraded, but the activity was only slightly greater than the level of significance. In addition, the effect was not reproducible and mutagenic activity was sometimes detected in control reactions in which DFP was omitted. Thus, we ascribe the activity to some kind of artifact and not to residual DFP. The DFP itself was mutagenic to strains TA100, TA1530, and TA1535 with and without activation. A dose-response effect was seen.

Related Compounds and Related Procedures

In principle, the procedure should work for all compounds having a P–F bond that can be hydrolyzed. However, many of these compounds are highly toxic (e.g., the nerve agents), and should only be handled in facilities offering high degrees of containment. A paper discusses the enzyme-catalyzed degradation of the nerve agents tabun, sarin, ethyl sarin, and soman.[24] In this paper the authors also note that solutions of these agents in pH 7.4 phosphate-buffered saline (PBS) could be decontaminated with 100 m*M* NaOH solution. The decontamination of the chemical warfare agents *O*-ethyl *S*-2-(diisopropylamino)ethyl methylphosphonothiolate (VX), 2,2′-dichlorodiethyl sulfide (mustard gas, mustard, S mustard, sulfur mustard, H, and HD), 2-propyl methylphosphonofluoridate (Sarin, GB), and 3,3-dimethyl-2-butyl methylphosphonofluoridate (Soman, GD) have been reviewed.[20, 25] Decontamination of these highly toxic compounds and their simulants (chemically similar, but less

toxic surrogates) continues to be an active field. See, for example,[26–30] and references contained within.

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DIMETHYL SULFATE AND RELATED COMPOUNDS

CAUTION! Refer to safety considerations section on page 9 before starting any of these procedures.

Dimethyl sulfate [DMS, sulfuric acid dimethyl ester, methyl sulfate, and $(CH_3)_2SO_4$] [77-78-1] is a clear, oily, high-boiling (bp 188 °C) liquid. This compound is quite volatile, has no characteristic odor,[1] is highly toxic,[2] and causes severe burns and injury to the lungs, kidneys, and liver. Dimethyl sulfate causes cancer in laboratory animals and maybe a human carcinogen.[3–8] This compound is slightly soluble in H_2O (2.8%) and is used as an alkylating agent industrially and in the laboratory.

Diethyl sulfate [DES, sulfuric acid diethyl ester, ethyl sulfate, and $(C_2H_5)_2SO_4$] [64-67-5] is also a volatile liquid (bp 209 °C) with a peppermint odor. This compound is almost insoluble in H_2O and is used as an alkylating agent industrially and in the laboratory. Diethyl sulfate causes cancer in experimental animals and may be a human carcinogen.[9, 10] Diethyl sulfate is a teratogen and severe skin irritant.[11]

Methyl methanesulfonate [MMS, methyl mesylate, methanesulfonic acid methyl ester, and $CH_3SO_2OCH_3$] [66-27-3] is a volatile liquid (bp 203 °C), soluble to the extent of about 1:5 in H_2O. Methyl methanesulfonate causes cancer in experimental animals.[12] Ethyl methanesulfonate (EMS, methanesulfonic acid ethyl ester, ethyl mesylate, and $CH_3SO_2OC_2H_5$) [62-50-0] is a volatile liquid (bp 213 °C), which is at least somewhat soluble in H_2O. Ethyl methanesulfonate causes cancer in experimental

Destruction of Hazardous Chemicals in the Laboratory, Fourth Edition. George Lunn and Eric B. Sansone.
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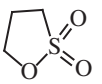
animals.[13] Methyl methanesulfonate[14] and ethyl methanesulfonate[15] are formed when methanesulfonic acid is allowed to react with methanol and ethanol, respectively. The reaction is retarded by the presence of water or a base.[14, 15] This reaction was, unfortunately, brought into prominence when the manufacturer inadvertently allowed ethanol and methanesulfonic acid to remain in contact for a prolonged period during the manufacture of Viracept (nelfinavir mesylate) tablets.[16] The resulting ethyl methanesulfonate contaminated the Viracept tablets at up to 2300 ppm but the contamination was not discovered until the tablets had been used by consumers. Subsequent work showed that ethyl methanesulfonate appears to exhibit a threshold effect for genotoxic activity.[17] Thus, no genotoxic activity is observed with low doses of ethyl methanesulfonate, but once the threshold is passed genotoxic activity increases with dose. Interestingly, *N*-ethyl-*N*-nitrosourea does not exhibit such a threshold effect. The implications of these findings remain controversial.

Butadiene diepoxide (**I**, BDE, erythritol anhydride, diepoxybutane, butadiene dioxide, Bioxiran, 2,2′-bioxirane, dianhydrothreitol, dianhydroerythritol, 1,1′-biethylene oxide, bp 56–58 °C at 25 mmHg) [298-18-0] is miscible with H_2O. This compound is used industrially, particularly in the polymer industry, and causes cancer in experimental animals.[18]

1,3-Propane sultone (**II**, PS, 1,2-oxathiolane 2,2-dioxide, 3-hydroxy-1-propanesulfonic acid sultone, mp 31–33 °C, bp 180 °C at 30 mmHg) [1120-71-4] is somewhat soluble in H_2O. This compound is used industrially, particularly in the detergent industry, and causes cancer in experimental animals.[19]

I



II

Although the toxicological properties of these compounds are not well known, by analogy with DMS they should be regarded as capable of causing lung injury and burns as well as being carcinogens. All of these compounds are mutagenic.

Principles of Destruction

Dimethyl sulfate is hydrolyzed by dilute base [sodium hydroxide (NaOH) solution (1 or 5 *M*), sodium carbonate (Na_2CO_3) solution (1 *M*), or ammonium hydroxide (NH_4OH) solution (1.5 *M*)] to methanol and methyl hydrogen sulfate.[20] Subsequent hydrolysis of methyl hydrogen sulfate to methanol and sulfuric acid is slow. We found, as others have,[21] that methyl hydrogen sulfate was nonmutagenic. Methyl hydrogen sulfate is a very poor alkylating agent.[22] When hydrolyzed using NH_4OH, the products are methylamine, dimethylamine, and trimethylamine. Hydrolysis destroyed DMS, a mutagenic compound, without producing other mutagenic species. The toxicity of

methyl hydrogen sulfate is not well established, so appropriate steps should be taken to protect workers handling this material. Similarly, DES, which is also mutagenic, can be hydrolyzed by the above reagents, although the process is slower.[23] The products are presumably analogous ethyl compounds. Ethanol is produced when the hydrolyzing agent is NaOH. Refluxing with alcoholic potassium hydroxide solution has also been reported to degrade dialkyl sulfates, but validation details were not provided.[24] Methyl and ethyl methanesulfonates can be hydrolyzed with either 1 or 5 *M* NaOH solution. The products are methanol or ethanol and, presumably, methanesulfonic acid. Methyl methanesulfonate and EMS are mutagenic, but methanesulfonic acid is not.[23] Butadiene diepoxide and 1,3-propane sultone are hydrolyzed with either 1 or 5 *M* NaOH solution.[23] Theoretically, 1,3-propane sultone could reform on acidification, although we could find no evidence for this. It is probably prudent, however, not to acidify the reaction mixtures when the reaction is complete. When NaOH was used the degradation efficiency was >99% in each case (see Table below). The compounds DMS, DES, MMS, and EMS can also be degraded using a 1 *M* solution of sodium thiosulfate ($Na_2S_2O_3$).[25] The degradation efficiency was >99.5%. The products of the reaction have not been determined.

A recent paper has demonstrated that DABCO (1,4-diazabicyclo[2.2.2]octane) can act as a scavenger and can remove alkyl methanesulfonates and alkyl benzenesulfonates from an organic solution.[26]

Compound	Molarity of NaOH (*M*)	Reaction Time (h)[a]	Residue (%)	Maximum Temperature rise (°C)	Products (%)	Product yield (%)
DMS	1	0.25*	<0.06	6	CH_3OH	68
	5	0.25*	<0.06	11	CH_3OH	71
DES	1	3*	<0.11	1	C_2H_5OH	47
	5	24	<0.11	1	C_2H_5OH	50
MMS	1	6	<0.09	1	CH_3OH	89
	5	2	<0.09	17	CH_3OH	65
EMS	1	48	<0.9	1	C_2H_5OH	88
	5	24	<0.9	1	C_2H_5OH	68
BDE	1	20	<0.4	1		
	5	22	<0.4	13		
PS	1	1	<0.24	3		
	5	1	<0.24	6		

Notes: For full details refer to the destruction procedures given above.
[a] Reaction time is normally measured from initial mixing. Reaction times marked with an asterisk were measured from the time the compound had completely dissolved in the NaOH solution.

Destruction Procedures

Destruction of Bulk Quantities of Dimethyl Sulfate and Diethyl Sulfate[20, 23]

Note: The following reaction times gave good results in our tests. However, the reaction time may be affected by factors, such as the size and shape of the flask and the rate of

stirring. If two phases are apparent, this is an indication that the reaction is not complete. Stirring should be continued until the reaction mixture is homogeneous.

To accomplish destruction 10 mL of DMS or DES was added at once to a flask containing 500 mL of rapidly stirred 1 *M* NaOH solution, 1 *M* Na_2CO_3 solution, or 1.5 *M* NH_4OH solution. No DMS could be detected 15 min after the last of the DMS went into solution, and no DES could be detected 3 h after the last of the DES went into solution. There was no apparent evolution of gas; the maximum temperature rise observed was 5 °C. At the end of the reaction, the mixture was neutralized, checked for completeness of destruction, and discarded.

This procedure may also be adapted for the destruction of larger quantities. Thus, 100 mL of DMS was added to 1 L of **5** *M* NaOH solution[27] and the reaction mixture was stirred. No DMS could be detected 15 min after the last of the DMS went into solution. Similar results were obtained for DES but dissolution was much slower. No DES could be detected in the solution 24 h after the addition of the DES to the base. The maximum temperature rise seen was 11 °C. At the end of the reaction, the mixture was neutralized, checked for completeness of destruction, and discarded.

Destruction of Bulk Quantities of Methyl Methanesulfonate, Ethyl Methanesulfonate, Butadiene Diepoxide, and 1,3-Propane Sultone[23]

1. To accomplish destruction 1 mL of the compound was added to 50 mL of 1 *M* NaOH solution and the reaction mixture was stirred for 1 h (PS), 6 h (MMS), 20 h (BDE), or 48 h (EMS). The reaction mixture was neutralized, checked for completeness of destruction, and discarded. The reaction times may vary depending on factors, such as the flask shape and the stirring rate. If the reaction mixture is not homogeneous, stirring should be continued until it is. The maximum temperature rise observed was 3 °C (for PS).
2. To accomplish destruction 1 mL of the compound was added to 10 mL of 5 *M* NaOH solution and the reaction mixture was stirred for 1 h (PS), 2 h (MMS), 22 h (BDE), or 24 h (EMS). The reaction mixture was neutralized, checked for completeness of destruction, and discarded. The reaction times may vary depending on factors, such as the shape of the flask and the stirring rate. If the reaction mixture is not homogeneous, stirring should be continued until it is. The maximum temperature rise observed was 17 °C (for MMS).

Destruction of Dimethyl Sulfate in Organic Solvents[20]

To accomplish destruction, 1 mL of a solution of DMS in methanol, ethanol, dimethylsulfoxide (DMSO), acetone, or *N,N*-dimethylformamide (DMF) (1 mL of DMS in 10 mL of solvent) was shaken with 4 mL of 1 *M* NaOH solution, 1 *M* Na_2CO_3 solution, or 1.5 *M* NH_4OH solution until the mixture was homogeneous. After 15 min, no DMS could be detected when the solvent was methanol, ethanol, DMSO, or DMF (<0.045%). After 1 h, no DMS could be detected when the solvent was acetone (<0.045%). The reaction mixture was neutralized, checked for completeness of destruction, and discarded.

For solvents not miscible with H_2O (toluene, *p*-xylene, benzene, 1-pentanol, ethyl acetate, chloroform, and carbon tetrachloride) 1 mL of a solution of DMS (1 mL of DMS in 10 mL of solvent) was added to 4 mL of 1 *M* NaOH, 1 *M* Na_2CO_3, or 1.5 *M* NH_4OH and the heterogeneous mixture was rapidly stirred. After 24 h no DMS could be detected in the organic layer (<0.045%).

Acetonitrile solutions can also be decontaminated using the methods described. Although the solution should be homogeneous, it was found that two layers were present in some instances. After 3 h the solutions were all homogeneous and no DMS could be detected (<0.045%). We recommend stirring for 24 h to ensure complete destruction. When the reaction was complete the reaction mixture was neutralized and the layers were separated, checked for completeness of destruction, and discarded.

Destruction of Dimethyl Sulfate, Diethyl Sulfate, Methyl Methanesulfonate, and Ethyl Methanesulfonate[25]

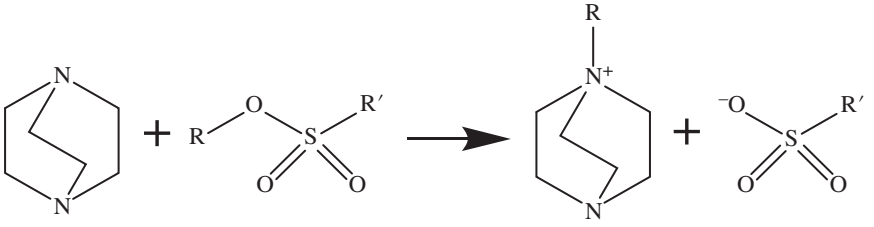
Bulk quantities of DMS, DES, MMS, or EMS were dissolved in acetone and for each 1 mL of this solution 49 mL of 1 *M* sodium thiosulfate solution was added. The mixture was stirred for 2 h, and discarded.

Spills of Dimethyl Sulfate[28]

The spill was covered with a 1:1:1 mixture of sodium carbonate or calcium carbonate, bentonite, and sand. The mixture was scooped up and added to a 10% NaOH solution. For each milliliter of DMS 10 mL of NaOH solution was used. This mixture was stirred for 24 h, checked for completeness of destruction, and discarded.

Removal of Alkyl Methanesulfonates and Alkyl Benzenesulfonates from Organic Solutions[26]

It has been found that DABCO (1,4-diazabicyclo[2.2.2]octane) can act as a scavenger and can remove alkyl methanesulfonates and alkyl benzenesulfonates from an organic solution. The reaction scheme is shown below.



R = Me, Et, n-Pr, iPr

R′ = methyl, phenyl

The quaternary product can be removed from solution using a water or a 2 *M* HCl wash. For example, 5 mM methyl methanesulfonate or ethyl methanesulfonate in 2-methylTHF that also contained 12.5 equivalents of DABCO was stirred at 50 °C. Removal of the alkylmethanesulfonate was 100% after 1 hour (MMS) or 24 hours (EMS). Water accelerated the removal.

Analytical Procedures[7, 29]

Note: If reaction mixtures that contain 5 *M* NaOH solution are to be analyzed, an aliquot of the reaction mixture should be diluted with four volumes of H_2O and 100 µL of this solution analyzed.

A 100 µL aliquot of the solution to be analyzed was added to 1 mL of a solution of 2 mL of acetic acid in 98 mL of 2-methoxyethanol. This mixture was swirled, and 1 mL of a solution of 5 g of 4- (4-nitrobenzyl)pyridine (4-NBP) in 100 mL of 2-methoxyethanol was added. The solution was heated at 100 °C for 10 min, then cooled in ice for 5 min. Piperidine (0.5 mL) and 2-methoxyethanol (2 mL) were added, and the violet color was determined at 560 nm using 10 mm disposable plastic cuvettes in a Gilford 240 UV/Vis spectrophotometer.

To check the efficacy of the analytical procedure, a small quantity of DMS can be added to the solution to be analyzed after the acetic acid-2-methoxyethanol has been added, but before the 4-NBP is added. A positive response will indicate that the analytical technique is satisfactory. Dichloromethane interfered with the determination, giving false positives. Although we have no reason to believe that DMS was not destroyed in dichloromethane solutions (and no mutagenic responses were obtained in these cases); we were not able to verify that complete destruction occurred. On the other hand, the false positives (obtained also in blank experiments) were small, with the largest value being <0.03% after 2 days. This result is equivalent to a destruction efficiency of >99.97%. We recommend carefully checking each layer of each reaction for completeness of destruction.

Using the analytical procedure described above, the limits of detection were DMS 10 mg/L, DES 27 mg/L, MMS 21 mg/L, EMS 275 mg/L, BDE 90 mg/L, and PS 66 mg/L, but these could easily be reduced by increasing the volume of reaction mixture tested. For example, using 150 µL of the solution containing EMS the limit of detection could be lowered to about 180 mg/L. Increasing the heating time to 60 min may also increase sensitivity.[30] We observed that a just noticeable violet color corresponded to a concentration that was about twice the detection limit given above, so the method could be used to rapidly screen a number of samples. The warming and cooling cycle may be inconvenient to carry out in many laboratories. For the analysis of DMS we found that it could be omitted, provided that the solution was allowed to stand for 4 h before the piperidine and 2-methoxyethanol were added. The appropriate blanks and positive controls should always be run. When diethylamine was substituted for piperidine no noticeable decrease in sensitivity was observed.

The products from these degradation reactions were determined by gas chromatography (GC) using a Hewlett Packard HP5880 gas chromatograph equipped with a

1.8-m × 2-mm i.d. column packed with 10% Carbowax 20 M + 2% KOH on 80/100 Chromosorb W AW with flame ionization detection. The oven temperature was 150 °C, the injection temperature was 200 °C, the detector temperature was 300 °C, and the carrier gas was nitrogen flowing at 30 mL/min. The approximate retention times were 0.5 min for methanol and 0.6 min for ethanol. The GC conditions given are only a guide, and the exact conditions would have to be determined experimentally.

A sensitive method using gas chromatography-mass spectrometry has been described.[31] The limit of quantitation is 1.0 ppm and the limit of detection is 0.3 ppm.

Driven perhaps by concern about trace amounts of genotoxic impurities in pharmaceuticals, a number of papers describing sensitive analytical methods have appeared in the literature. By analogy with methyl methanesulfonate the structurally similar alkyl benzenesulfonates and alkyl p-toluenesulfonates have also come under suspicion.

Technique	Analyte	LOQ	LOD	Reference
CE	Dimethyl sulfate	1.0 µg/mL	0.3 µg/mL	31
CE	Chloroacetyl chloride	1.0 µg/mL	0.3 µg/mL	31
LC-MS	Methyl, ethyl, propyl, isopropyl methanesulfonate	10 ng/mL	4 ng/mL	32
	Methyl, ethyl, propyl, isopropyl benzenesulfonate	5 ng/mL	2 ng/mL	32
	Methyl, ethyl, propyl, isopropyl p-toluenesulfonate	5 ng/mL	2 ng/mL	32
LC-MS with derivatization	Dimethyl sulfate	0.05 µg/mL	NA	33
HPLC with refractive index detection	Butadiene diepoxide	NA	NA	34
GC-MS	Dimethyl sulfate	0.48 mg/kg	0.24 mg/kg	35
Mass spectrometry	Methyl p-toluenesulfonate	NA	0.1 ppm	36

NA: Not available.

Mutagenicity Assays

The mutagenicity assays were carried out as described on page 4. For DMS, tester strain TA100 was used, and for DES, MMS, EMS, BDE, and PS tester strains TA98, TA100, TA1530, and TA1535 were used. For each plate, 100 µL of solution (corresponding to 2.6 mg of undegraded DMS, 2.3 mg of DES, 2.5 mg of MMS, 2.3 mg of EMS, 2.0 mg of BDE, and 2.5 mg of PS) was used.[23]

Solutions of DMS in various solvents were degraded using 1 *M* NaOH solution, 1 *M* Na_2CO_3 solution or 1.5 *M* NH_4OH solution, and the reaction mixtures were tested for mutagenicity. No mutagenic response was observed when solutions of DMS in methanol, H_2O, acetone, and DMSO were tested after 2 h of reaction, when the organic layer of solutions of DMS in dichloromethane and benzene were tested after 24 h of reaction, or when the organic layer of a solution of DMS in toluene was tested after 3 days of reaction.

The reaction mixture was cytotoxic (and therefore, a determination of mutagenicity could not be made) when solutions of DMS in methanol or acetone were degraded using 1 *M* NaOH and when solutions of DMS in 1-pentanol were degraded using 1 *M* NaOH, 1 *M* Na_2CO_3, or 1.5 *M* NH_4OH.

A solution of DMS in ethanol was allowed to stand and gave a strong mutagenic response that slowly decreased with time, presumably as the DMS degraded. A similar decrease in activity was observed when a solution of DMS in ethanol was measured colorimetrically, as described above.

No mutagenic activity was observed when DES, MMS, EMS, BDE, or PS were degraded with NaOH as described using the reaction times specified above with the exception that the degradation of BDE with 1 *M* NaOH solution gave reaction mixtures that were just mutagenic to TA98 without activation (59 revertants observed and control value 27). Degradation of BDE with 5 *M* NaOH did not give reaction mixtures that were significantly mutagenic.

Tester strains TA97, TA98, TA100, and TA102 were used when DMS, DES, MMS, and EMS were degraded using sodium thiosulfate solution.[25] No mutagenic activity was found.

In another paper[38] DMS, DES, MMS, EMS, and a variety of related dialkyl sulfates and alkyl sulfonic acid esters have been shown to be genotoxic in an *in vitro* micronucleus assay and a deletion recombination assay.[38]

Related Compounds

These destruction procedures and analytical methods should be applicable to other dialkyl sulfates, alkyl methanesulfonates, and related compounds, although we have not verified this. Problems might arise when large alkyl groups are present because the compound may not be miscible with H_2O. In addition, large alkyl groups may slow hydrolysis to such an extent that complete destruction may not be obtained. In the work described above, the hydrolysis slowed as the size of the alkyl group increased. β-Propiolactone, which is a cyclic ester, was completely degraded using these methods, although there did seem to be some tendency to reform β-propiolactone on acidification and mutagenic reaction mixtures were produced, so these procedures cannot be recommended for this compound. See the β-propiolactone monograph for details of a destruction procedure for this compound.

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