NMR Sample Policy

Version: 20 Jul 2006

Task: ***NMR Sample Preparation***	
Equipment Used: Centrifuge, pH meter, NMR spectrometers, fumehood, fridges, freezers, tube cleaner	***Location***: Rooms G14, G16, G23 and G24 in the Henry Wellcome Building for Biomolecular NMR Spectroscopy, University of Birmingham
Hazards (e.g. physical, ergonomic, biological, chemical, radiation):	***Risk Assessment*** ('high', 'medium' or 'low'):
1. **Radiation**: danger to people and equipment due to inappropriate submission of radioactive samples.	1. Low: All Users must understand and agree that no samples are allowed that require any form of radiological monitoring
2. **Chemical**: danger to people and equipment due to submission of carcinogenic, corrosive, flammable or toxic samples.	2. Medium: Use of hazardous chemicals is discouraged. Users must follow all Control of Substances Hazardous to Health (COSHH) regulations. Hazardous chemicals brought into the building must be reported and approved by HWB•NMR staff, and labelled and stored in a safe manner at all times. Excluded Chemical Weapons Convention Scheduled Chemicals are listed in Appendix 2. COSHH Assessment NMR002 'Protein NMR Sample Preparation' on the Cancer Studies COSHH database covers a number of chemicals commonly used in biomolecular NMR. Copies are available from S. Rhodes.
3. **Biological**: danger to people due to submission of biological hazards such as toxins, infectious agents, viruses, pathogenic bacteria.	3. Low: Use of any biological hazard is discouraged and must follow all COSHH regulations. Some of the disallowed biological agents are listed in Appendix 1. All samples must be labelled and stored in a safe manner at all times.
4. **Ethical**: samples submitted may be or human or animal origins, requiring consent.	4. Low: No testing of human or animal subjects shall be conducted on the premises. Ethical approvals for human or animal tissues or biofluids must be obtained from the approproate ethical review body and submitted to HWB•NMR staff prior to initiation of NMR analysis.
5. **Physical**: Inappropriate use of manual centrifuge can cause sample breakage and debris, and involves spinning an exposed rotor. The insertion of samples into NMR magnets requires climbing access platform near the magnets.	5. Low: Use of equipment in the laboratory and NMR chambers requires appropriate user induction and appropriate access systems.
6. **Financial** (e.g. damage to NMR systems): NMR sample tubes can break and release their contents onto and damage probes. Probes can be damaged by insertion of hazardous samples, stripping the probe tuning rods by forced overturning, or use of excessive RF pulse power or probe temperatures. These probes can cost over £100000, and full insurance cover is not affordable.	6. Medium: NMR samples can only be inserted and experiments initiated by fully trained Users and Staff who understand the risks and accept their responsibilities. Users are not permitted to modify the NMR hardware or control software or attempt new pulse sequences unless these actions have been authorized by the NMR staff.
Safety Pre-Requisites (e.g. secure access, training, work order, supervision, warning signs, protective equipment): 1. Ensure compliance with COSHH regulations (see http://www.coshh-essentials.org.uk/) 2. Submit completed form for NMR time or sample analysis indicating all hazards. Provide MSDS datasheets for any hazardous chemicals. 3. All Users must obtain approval from the Operations Manager before initiating NMR experiments, including the completion of an orientation for the safe use of the NMR systems.	Prepared by 1.  Prof Michael Overduin, phone 4143802, M.Overduin@bham.ac.uk 2.  Dr Ulrich Günther, phone 4148361, U.L.Gunther@bham.ac.uk

Key Points (warning, check points, emergency/first aid information):

1. In case of any accident, concern or question regarding these protocols please notify the Preparers (see above)
2. It is the user's responsibility to be familiar with and comply with these procedures.
3. Negligence or non-compliance can result in barring from future use of HWB-NMR resources.
4. Disputes will be handled by the Health and Safety Committee.
5. Approved chemicals (e.g. $^{2}H_2O$) and glassware (e.g. NMR tubes) may be stocked in the HWB•NMR sample preparation laboratory, and are available upon request from Sue Rhodes.

Considerations for Protein NMR Sample Preparation

1. **Molecular weight**: Larger molecules have longer correlation times, leading to faster relaxation and increased line-widths. NMR is best suited to characterizing folded proteins in the 5-40 kDa range, larger proteins and complexes require exponentially more time and expense. Tags used for affinity purification (e.g. glutathione S transferase or thioredoxin) should be cleaved and removed unless they are small (under 10 residues, e.g. His-tags).

2. **Concentration**: Protein concentrations should ideally be between 0.5 and 1.5 mM for structural analysis (1mM = 10mg/ml for a 10kDa protein), noting that doubling the concentration requires approximately a quarter of the acquisition time to obtain a given signal to noise ratio, but increases sample viscosity. Proteins should be purified to >90% homogeneity, i.e. a single clean band on a SDS-PAGE gel or, even better, a single peak on a size exclusion chromatography column. The sequence and exact size of the construct should be verified by DNA or protein sequencing and mass spectrometry, respectively. Ideally the monomeric (or oligomeric state) should be demonstrated by size exclusion chromatography, dynamic light scattering, analytical ultracentrifugation or other analytical measure.

3. **Stability**: The sample may be in the NMR spectrometer for many hours or days, with temperatures for data collection ranging from 4 - 40ºC. The standard operating temperature is 25ºC. Highly concentrated proteins tend to aggregate and precipitate, yielding poor NMR spectra.

4. **Buffer**: Ideally 20 mM, although 0-50 mM is common. Phosphate buffer is an economical non-protonated buffer, and there also are a variety of perdeuterated buffers (d-Tris, d-HEPES) available that do not obscure protein NMR signals.

5. **pH**: Usually acidic pH is required (typically 5-7) because many NMR experiments require observation of exchangeable amide protons which are difficult to observe at higher pH. Protein structure and interactions can be pH sensitive, so the final sample pH should be verified.

6. **Ionic Strength**: Salt (e.g. KCl) can increase protein solubility. However, high ionic strength (>150mM) demands longer 90º pulses, increases sample heating, reduces signal to noise (especially using cryogenic probes) and makes it more difficult to tune the probe.

7. **Paramagnetics**: Paramagnetic metals such as Cu(II), Mn(II), Cr(III), Fe(III) and Co(II) lead to NMR line broadening, and should typically be avoided.

8. **Volume**: Each NMR sample should consist of a final volume of 550 μL, including all additives, for a standard tube (e.g. Wilmad 535).

9. **Additives**: $^{2}H_2O$ is added to 10% (or 5% for high value samples) for locking on the NMR signal frequencies, unless a 100% $^{2}H_2O$ solution is being used to clearly observe nonexchangeable protons. Sodium azide (usually 1-3mM) is added to prevent microbial contamination. A reducing agent such as DTT (<1mM or higher concentration - 30mM - if perdeuterated DTT) can be added to prevent protein oxidation. The internal standard 2,2-dimethylsilapentane-5-sulfonic acid (DSS) can be added (typically 50 μM) to reference the chemical shifts. If sample gets proteolysed, protease inhibitors can be added at low concentrations (<50μM)

10. **Sample Tubes**: Wilmad 535 NMR tubes (7 inch) are standard for protein NMR. New NMR tubes are not 'analytically clean' when delivered, but usually have organic or inorganic residues. Ensure tubes are clean (a rinse with water or buffer is advisable) and not chipped or warped by excessive heat. Shigemi tubes can be used for low volume samples, but require special care to eliminate bubbles. Tubes should be capped and the cap parafilmed to avoid evaporative loss.

11. **Isotope labelling**: Although proteins can initially be assessed for suitability for structural analysis in unlabelled forms, detailed studies require labelling with ^{15}N for small (~50-100 residues), ^{15}N and ^{13}C for medium (~100-150 residues), and ^{15}N, ^{13}C and ^{2}H for large (>~150 residues) proteins.

Procedure – Operational Notes

1. **Use of concentrators**: Proteins are often exchanged into their final solution conditions using concentrators with molecular weight cut off filters. The filters are stored with glycerol, which must be removed by at least three washes or spins of the concentrator with deionized water.
2. **Washing NMR tubes**: NMR tube washers or Solvent Jet Cleaners can be purchased from GPE Limited, Sigma Aldrich and Wilmad, an economical 9 tube washer unit can be built by the School of Chemistry glassblowing shop, and will be provided by the HWB-NMR wet-lab and Overduin's laboratory (Institute for Cancer Studies S313). Strong acids such as Nochromix (Godax Laboratories) are available to remove adhered materials and deposits by overnight soaking, followed by washes with water or buffer.
3. **Transport of NMR tubes**: Individual NMR tubes can be safely transported in NMR tube racks made by, for example, Kimble-Kontes or Wilmad, graduated cylinders, or inverted and taped 15 mL Falcon tubes.
4. **Short term storage of NMR tubes**: use specialized NMR tube racks at the consoles and refrigerator to minimize

risks of breakage of the delicate NMR tubes.

5. **Long term storage of NMR tubes**: We recommend flash freezing proteins in liquid nitrogen and storage at -80°C to minimize risks of oxidation or degradation. A small scale freeze and thaw trial experiment is advised to assess the risk of protein precipitation during sample warm up.

Sources and references

The following companies sell NMR reagents and consumables:

1. Spectra Gases - http://www.spectra-gases.com sells isotope labelled reagents for NMR
2. Cambridge Isotope Laboratories - http://www.isotope.com sells isotope labelled reagents for NMR
3. Isotec - (a division of Sigma/Aldrich) sells isotope labelled reagents for NMR
4. Silantes GmbH - http://www.silantes.com sells isotope labelled reagents for NMR
5. C/D/N ISOTOPES - http://www.cdniso.com sells isotope labelled reagents for NMR
6. Medical Isotopes, Inc. - http://www.medicalisotopes.com sells isotope labelled reagents for NMR
7. Wilmad Glass Company: The standard NMR tube is Wilmad product 5mm 535-PP 7, although a less expensive tube (528-PP 7) can also be used for routine use.
8. Shigemi, Inc: For low volume samples use 5 mm tubes from Shigemi, Inc (412-444-3011). Ensure that tubes are matched to the appropriate solvent.
9. GPE Limited http://www.gpelimited.co.uk/ sells glassware for NMR
10. Godax Laboratories sells Nochromix

The following articles may provide useful information:

Chemical shift standards

DS Wishart et al. (1995) 1H, 13C and 15N chemical shift referencing in biomolecular NMR, J Biomol NMR 6, 135-140.

NMR Buffers

CH Schein (1990) Solubility as a function of protein structure and solvent components, Biotechnology 8, 308-316

J Freund and HR Kalbitzer (1995) Physiological buffers for NMR spectroscopy, J Biomol NMR 5, 321-322.

General NMR

Three Methods in Enzymology volumes (176, 177 and 239) are dedicated to biomolecular NMR. See Norman Oppenheimer's article "Sample Preparation" pp 78-89, Vol 176 for useful hints on preparing a NMR sample.

Protein solubility

AP Golovanov et al. A simple method for improving protein solubility and long-term stability. J Am Chem Soc. 2004 126:8933-9.

Appendix 1. Excluded Infectious Agents, Bacteria, Viruses and Toxins:

Note: The following list of excluded substances is not necessarily exhaustive. Please contact NMR staff if you are unsure about a particular substance.

ANTI-TERRORISM, CRIME AND SECURITY ACT 2001

Part 7 of the Anti-Terrorism, Crime and Security Act 2001 is concerned with the security of dangerous substances that may be targeted or used by terrorists. These substances are listed in Schedule 5 of the Act. The Schedule was amended in 2007. The current list is shown below and includes viruses, rickettsiae, fungi, bacteria and toxins. The toxins are also included in HAZDAT.

The provisions set out in Part 7 (and Schedules 5 and 6) place an obligation on managers of laboratories and other premises holding stocks of specified disease-causing micro-organisms and toxins to notify their holdings, and to comply with any reasonable security requirements which the police may impose.

It also requires managers of laboratories and other premises, on request, to furnish the police with details of people with access to the dangerous substances held there. The Secretary of State is given power to direct that a named individual must not be allowed access to such disease strains or the premises in which they are held.

Reporting etc, is co-ordinated through the University Health and Safety Unit.

VIRUSES

Chikungunya virus

Congo-crimean haemorrhagic fever virus

Mobala virus

Monkey pox virus

Dengue fever virus

Dobrava/Belgrade virus

Eastern equine encephalitis virus

Ebola virus

Everglades virus

Getah virus

Guanarito virus

Hantaan virus

Hendra virus (Equine morbillivirus)

Herpes simiae (B virus)

Influenza viruses (pandemic strains)

Japanese encephalitis virus

Junin virus

Kyasanur Forest virus

Lassa fever virus

Louping ill virus

Lymphocytic choriomeningitis virus

Machupo virus

Marburg virus

Mayaro virus

Middleburg virus

Mucambo virus

Murray Valley encephalitis virus

Ndumu virus

Nipah virus

Omsk haemorrhagic fever virus

Polio virus

Powassan virus

Rabies virus

Rift Valley fever virus

Rocio virus

Sabia virus

Sagiyama virus

Sin Nombre virus

St Louis encephalitis virus

Tick-borne encephalitis virus (Russian Spring-Summer encephalitis virus)

Variola virus

Venezuelan equine encephalitis virus

West Nile fever virus.

Western equine encephalitis virus

Yellow fever virus

RICKETTSIAE

Coxiella burnetii

Rickettsia prowazeki

Rickettsia rickettsii

Rickettsia typhi (mooseri).

BACTERIA

Bacillus anthracis

Brucella abortus

Brucella canis

Brucella melitensis

Brucella suis

Burkholderia mallei (Pseudomonas mallei)

Burkholderia pseudomallei (Pseudomonas pseudomallei)

Chlamydophila psittaci

Clostridium botulinum

Clostridium perfringens

Enterohaemorrhagic Escherichia coli, serotype 0157 and verotoxin producing strains

Francisella tularensis

Multiple-drug resistant Salmonella paratyphi

Mycobacterium tuberculosis

Salmonella paratyphi A, B, C

Salmonella typhi

Shigella boydii

Shigella dysenteriae

Shigella flexneri.

Vibrio cholerae

Yersinia pestis

FUNGI

Cladophialophora bantiana

Cryptococcus neoformans."

TOXINS

TOXIN	***CAS NUMBER***
Abrin Modeccin toxin Abrin	1393-62-0
Botulinum toxins Botulin D	93384-46-4
Botulinum toxin A	93384-43-1
Botulinum toxin B	
Botulinum toxin F	
Clostridium botulinum toxin	
Clostridium perfringens toxins Clostridium botulinum neurotoxin	
Clostridium perfringens, epsilon toxin	
Clostridium perfringens, type A enterotoxin	
Conotoxin Conotoxin	123210-68-4
Modeccin	65988-88-7

Modeccin	
Ricin Ricin	9009-86-3
Saxitoxin Saxitoxin	35523-89-8
Shiga and Shiga-like toxins Shiga toxin	
DNA (Escherichia coli strain KY-019 clone pKTN1054 Shiga-like toxin SLT-II subunit A gene plus Shiga-like toxin SLT-II subunit B gene)	153834-56-1
DNA (Escherichia coli strain KY-019 clone pKTN1054 Shiga-like toxin SLT-II subunit A gene)	153834-58-3
DNA (Escherichia coli strain KY-019 clone pKTN1054 Shiga-like toxin SLT-II subunit B gene)	153834-60-7
DNA (Escherichia coli strain TK-051 clone pKTN1050 Shiga-like toxin SLT-II subunit A gene plus Shiga-like toxin SLT-II subunit B gene)	153834-57-2
DNA (Escherichia coli strain TK-051 clone pKTN1050 Shiga-like toxin SLT-II subunit A gene)	153834-59-4
Verotoxin 1 (Shiga shigella B subunit)	620190-09-2
Staphylococcal enterotoxins Staphylococcal enterotoxin A (Staphylococcus aureus gene SEA)	915245-87-3
Staphylococcal enterotoxin B (Staphylococcus aureus aureus strain COL gene seb)	811333-16-1
Staphylococcal enterotoxin C-bovine (Staphylococcus aureus host cattle gene sec-bov)	349587-80-0
Staphylococcal enterotoxin E (Staphylococcus aureus)	197981-85-4
Tetrodotoxin Tetrodotoxin	4368-28-9
Viscum Album Lectin 1 (Viscumin) Viscum Album Lectin 1	83590-17-4
Volkensin toxin Volkensin toxin	91933-11-8

Any reference to a micro-organism or toxin includes:

(a) any genetic material containing any nucleic acid sequence
- associated with the pathogenicity of the micro-organism or
- for the coding of the toxin; and

(b) any genetically modified organism containing any such sequence.

Any reference to a toxin includes subunits of the toxin.

Abrin Aflatoxins African horsesickness virus African swine fever	Marburg virus Menangle virus Mycoplasma capricolum /M. F38/M. mycoides capri (contagious caprine

Akabane virus	Mycoplasma mycoides mycoides (contagious bovine
Avian influenza (highly pathogenic)	pleuropneumonia)
Bacillus anthracis	Newcastle disease virus (exotic)
Bluetongue virus (exotic)	Nipah virus
Botulinum toxins	Peronosclerospora philippinensis
Bovine spongiform encephalopathy agent	Peste des petits ruminants
Brucella abortus,	Phakopsora pachyrhizi
Brucella melitensis	pleuropneumonia)
Brucella suis	Plum pox potyvirus
Burkholderia (Pseudomonas) mallei	Prion
Burkholderia (Pseudomonas) pseudomallei	Ralstonia solanacearum Race 3
Camel pox virus	Ricin
Classical swine fever	Rickettsia prowazekii
Clostridium botulinum	Rickettsia rickettsii
Clostridium perfringens epsilon toxin	Rift Valley fever virus
Coccidioides immitis	Rinderpest virus
Conotoxins	Saxitoxin
Cowdria ruminantium (heartwater)	Sclerophthora rayssiae var. zeae
Coxiella burnetii	Sheep pox
Crimean-Congo haemorraghic fever virus	Shigatoxin
Diacetoxyscerpinol	South American haemorraghic fever viruses
Eastern equine encephalitis virus	Staphylococcal enterotoxins
Ebola viruses	Swine vesicular disease virus
Equine morbillivirus (Hendra virus)	Synchytrium endobioticum
Foot-and-mouth disease virus	T-2 toxin
Francisella tularensis	Tetrodotoxin
Goat pox virus	Tick-borne encephalitis complex viruses
Japanese encephalitis virus	Variola major virus (smallpox)
Lassa fever virus	Venezuelan equine encephalitis virus
Liberobacter africanus	Vesicular stomatitis (exotic)
Liberobacter asiaticus	Viruses causing hantavirus pulmonary syndrome
Lumpy skin disease virus	Xanthomonas oryzae pv. oryzicola
Malignant catarrhal fever	Xylella fastidiosa (citrus variegated chlorosis strain)
	Yellow fever virus
	Yersinia pestis

Appendix 2: Excluded chemicals:
Chemical Weapons Convention Scheduled Chemicals.

The following Schedules list toxic chemicals and their precursors.
(Whenever reference is made to groups of dialkylated chemicals, followed by a list of alkyl groups in parentheses, all chemicals possible by all possible combinations of alkyl groups listed in the parentheses are considered as listed in the respective Schedule as long as they are not explicitly exempted.)

Schedule 1

A. Toxic chemicals:

	CAS registry number
(1) O-Alkyl (<C10, incl. cycloalkyl) alkyl(Me, Et, n-Pr or i-Pr)-phosphonofluoridates	
e.g. Sarin: O-Isopropyl methylphosphonofluoridate	107-44-8
Soman: O-Pinacolyl methylphosphonofluoridate	96-64-0
(2) O-Alkyl (<C10, incl. cycloalkyl) N,N-dialkyl (Me, Et, n-Pr or i-Pr) phosphoramidocyanidates	
e.g. Tabun: O-Ethyl N,N-dimethylphosphoramidocyanidate	77-81-6
(3) O-Alkyl (H or <C10, incl. cycloalkyl) S-2-dialkyl(Me, Et, n-Pr or i-Pr)-aminoethyl alkyl (Me, Et, n-Pr or i-Pr) phosphonothiolates and corresponding alkylated or protonated salts	
e.g. VX: O-Ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate	50782-69-9
(4) Sulfur mustards:	
2-Chloroethylchloromethylsulfide	2625-76-5
Mustard gas: Bis(2-chloroethyl)sulfide	505-60-2
Bis(2-chloroethylthio)methane	63869-13-6
Sesquimustard: 1,2-Bis(2-chloroethylthio)ethane	3563-36-8
1,3-Bis(2-chloroethylthio)-n-propane	63905-10-2
1,4-Bis(2-chloroethylthio)-n-butane	142868-93-7
1,5-Bis(2-chloroethylthio)-n-pentane	142868-94-8
Bis(2-chloroethylthiomethyl)ether	63918-90-1
O-Mustard: Bis(2-chloroethylthioethyl)ether	63918-89-8
(5) Lewisites:	
Lewisite 1: 2-Chlorovinyldichloroarsine	541-25-3
Lewisite 2: Bis(2-chlorovinyl)chloroarsine	40334-69-8

Lewisite 3: Tris(2-chlorovinyl)arsine	40334-70-1
(6) Nitrogen mustards:	
HN1: Bis(2-chloroethyl)ethylamine	538-07-8
HN2: Bis(2-chloroethyl)methylamine	51-75-2
HN3: Tris(2-chloroethyl)amine	555-77-1
(7) Saxitoxin	35523-89-8
(8) Ricin	9009-86-3

B. Precursors:

(9) Alkyl (Me, Et, n-Pr or i-Pr) phosphonyldifluorides	
e.g. DF: Methylphosphonyldifluoride	676-99-3
(10) O-Alkyl (H or <C10, incl. cycloalkyl) O-2-dialkyl (Me, Et, n-Pr or i-Pr)-aminoethyl alkyl (Me, Et, n-Pr or i-Pr) phosphonites and corresponding alkylated or protonated salts	
e.g. QL: O-Ethyl O-2-diisopropylaminoethylmethylphosphonite	57856-11-8
(11) Chlorosarin: O-Isopropyl methylphosphonochloridate	1445-76-7
(12) Chlorosoman: O-Pinacolyl methylphosphonochloridate	7040-57-5

Schedule 2

A. Toxic chemicals:

	CAS registry number
(1) Amiton: O,O-Diethyl S-[2-(diethylamino)ethyl]phosphorothiolate and corresponding alkylated or protonated salts	78-53-5
(2) PFIB: 1,1,3,3,3-Pentafluoro-2-(trifluoromethyl)-1-propene	382-21-8
(3) BZ:3-Quinuclidinyl benzilate (*)	6581-06-2

B. Precursors:

(4) Chemicals, except for those listed in Schedule 1, containing a phosphorus atom to which is bonded one methyl, ethyl or propyl (normal or iso) group but not further carbon atoms,	
e.g. Methylphosphonyl dichloride	676-97-1
Dimethyl methylphosphonate	756-79-6
Exemption: Fonofos: O-Ethyl S-phenylethylphosphonothiolothionate	944-22-9
(5) N,N-Dialkyl (Me, Et, n-Pr or i-Pr) phosphoramidic dihalides	
(6) Dialkyl (Me, Et, n-Pr or i-Pr) N,N-dialkyl (Me, Et, n-Pr or i-Pr)-phosphoramidates	
(7) Arsenic trichloride	7784-34-1
(8) 2,2-Diphenyl-2-hydroxyacetic acid	76-93-7
(9) Quinuclidin-3-ol	1619-34-7
(10) N,N-Dialkyl (Me, Et, n-Pr or i-Pr) aminoethyl-2-chlorides and corresponding protonated salts	
(11) N,N-Dialkyl (Me, Et, n-Pr or i-Pr) aminoethane-2-ols and corresponding protonated salts	
Exemptions: N,N-Dimethylaminoethanol and corresponding protonated salts	108-01-0
N,N-Diethylaminoethanol and corresponding protonated salts	100-37-8
(12) N,N-Dialkyl (Me, Et, n-Pr or i-Pr) aminoethane-2-thiols and corresponding protonated salts	
(13) Thiodiglycol: Bis(2-hydroxyethyl)sulfide	111-48-8
(14) Pinacolyl alcohol:3,3-Dimethylbutan-2-ol	464-07-3

Schedule 3

A. Toxic chemicals:

	CAS registry number
(1) Phosgene: Carbonyl dichloride	75-44-5
(2) Cyanogen chloride	506-77-4
(3) Hydrogen cyanide	74-90-8
(4) Chloropicrin: Trichloronitromethane	76-06-2

B. Precursors:

(5) Phosphorus oxychloride	10025-87-3
(6) Phosphorus trichloride	7719-12-2
(7) Phosphorus pentachloride	10026-13-8
(8) Trimethyl phosphite	121-45-9
(9) Triethyl phosphite	122-52-1
(10) Dimethyl phosphite	868-85-9
(11) Diethyl phosphite	762-04-9

(12) Sulfur monochloride	10025-67-9
(13) Sulfur dichloride	10545-99-0
(14) Thionyl chloride	7719-09-7
(15) Ethyldiethanolamine	139-87-7
(16) Methyldiethanolamine	105-59-9
(17) Triethanolamine * Please just inform us if you intend to use TEA	102-71-6