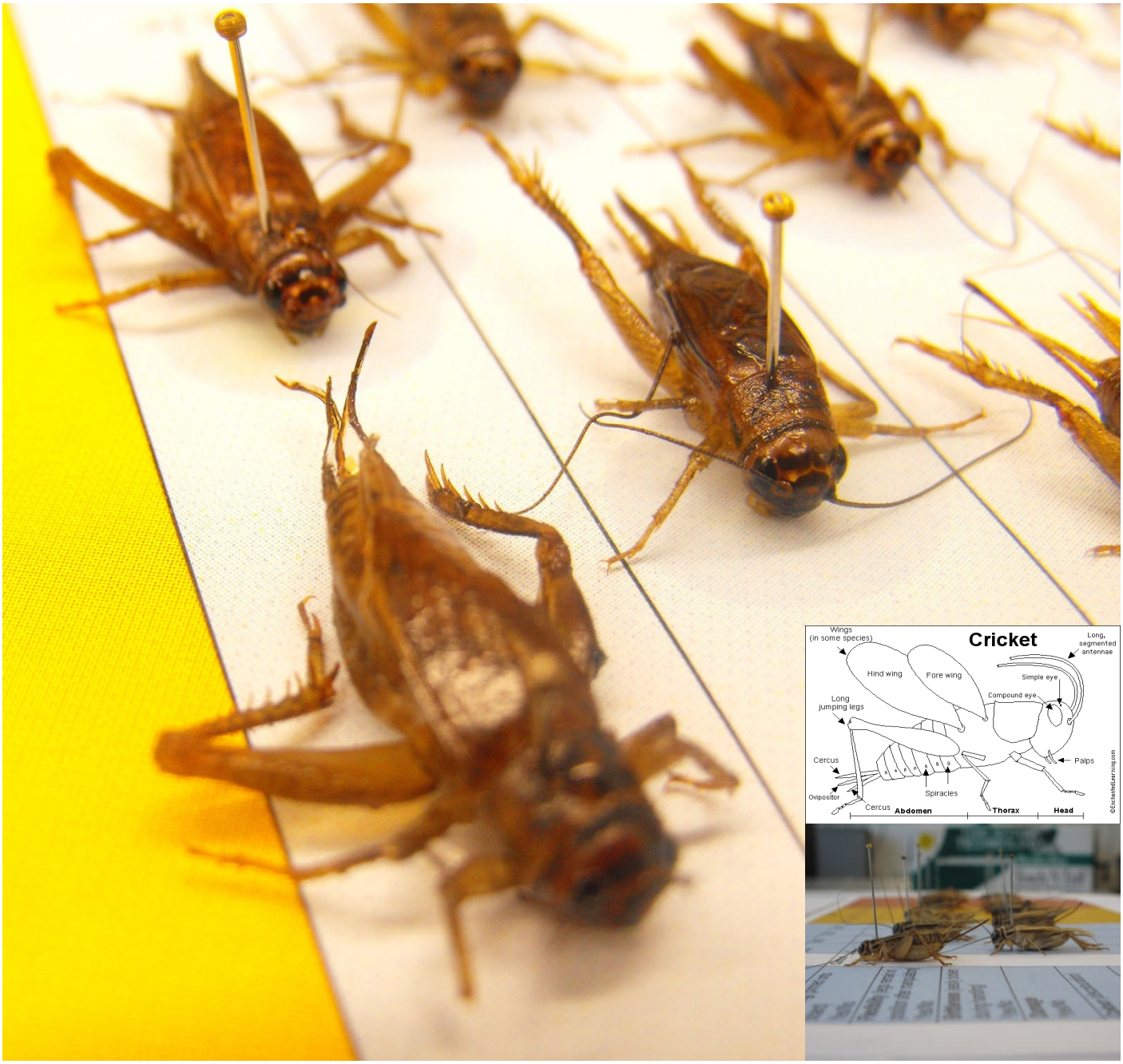

Development of Ethically Acceptable Techniques for Invertebrate Wet-Pit Trapping

PROJECT SUMMARY 1.0

February 2011

TITLE: Development of ethically-acceptable techniques for invertebrate wet pit trapping

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Outcomes:
Animal Ethics: Chemical solutions that ensure rapid death for target (invertebrates) and non-target (vertebrates) species falling into wet pit solution. This should occur with as little discomfort and suffering as possible.
Specimen Preservation: Preservation of specimens to a standard suitable for taxonomic research (maintains visible morphological characters)
DNA Preservation: Preservation of genetic material suitable for molecular examination
Preservative Stability: Solution stable for extended periods under extreme climatic conditions (eg. exposed to elements in Pilbara or Kimberly summer for up to three months).
Occupational Health and Safety: Minimal personal protective equipment should be needed for safe use (ie. face shield in ventilated environment with gloves) without health risk

Animal Ethics

Chemical solutions that ensure rapid death for target (invertebrates) and non-target (vertebrates) species falling into a wet pit solution. This should occur with as little discomfort and suffering as possible.

Table 1: Chemicals commonly used in wet pit traps

Preservative	Comment
Ethylene glycol	Anti freeze, hygroscopic, phased out because of safety and environmental concerns, toxic, good for spiders, risk of wildlife damage to wetpit trap (density = 1.113)
Propylene glycol	Highly referenced, antifreeze, hygroscopic, GRAS material, replacement for ethylene glycol, risk of wildlife damage to wetpit trap, good for spiders, unknown effects on DNA quality (density =1.036; BP = 188; VP = 0.129 @ 25^0C)
Ethanol	Highly referenced, especially on DNA preservation, evaporates readily (density = 0.789; BP = 78.5; VP = 67.5 @ 20^0C)
Propanol	Non-toxic alternative to formaldehyde (density = 0.786)
Formaldehyde	Not recommended for DNA, Group 1 carcinogen, safety issues
Brine	Poor preservative properties
Methanol	Used sporadically (density = 0.791)
Ethylamine	Used sporadically (density = 0.683)
Glycerol	Softens tissue over time (density = 1.263)
Borax solution	Poor preservative properties
White vinegar	Short term only
FAACC	Formaldehyde, acetic acid, calcium chloride, preserves internal reproductive organs
Phosphate formaldehyde	Preserves internal reproductive organs
Water	No preservative properties (density = 1.00; BP = 100; VP = 17.5 @ 20^0C)

A desktop survey and advice from entomologists (DAFWA and experts) show that various chemicals have been used as killing/curative agents (see in Table 1). Target species which fall into the trap are killed by drowning and fixation in the “preservative” solution and this was achieved using ethylene glycol. For many years, ethylene glycol remained the staple preservative until the realisation that it was toxic to fauna and poses a risk to the environment. As recent as 2010, it was used in a survey that assessed short-range endemic (SRE) invertebrates for the Fortescue Metals Group. Its replacement, propylene glycol, is generally regarded as safe (GRAS, USFDA). Currently, propylene glycol and ethanol are the most referenced killing and preserving agents. Both are the primary focus of this project

though, as can be seen in Table 1, each has significantly contrasting physical and chemical properties.

Specimen Preservation:

Preservation of specimens to a standard suitable for taxonomic research (maintains visible morphological characters)

Table 2: Morphological characteristics

Handling conditions	Visible morphological characters
Mounting	Is the thorax sufficiently rigid enabling insertion of 40mm pin through to mounting board
Flexibility	Can legs and wings be folded into position
Brittleness	Are wings, limbs, antennae, circa, palps, easily broken during handling
Damage	Are appendages semi- or completely dislodged at the joints
Colour	Are there signs of discolouration
Preservative	Any leaching of pigmentation causing discolouration of preservative
Odour	Other than that from ethanol, signs of spoilage and/or cloudiness
Swelling	Indications of oedema, disfigurement of head, thorax, abdomen, limbs
Shrinkage	Signs of disfigurement, dehydration or collapse

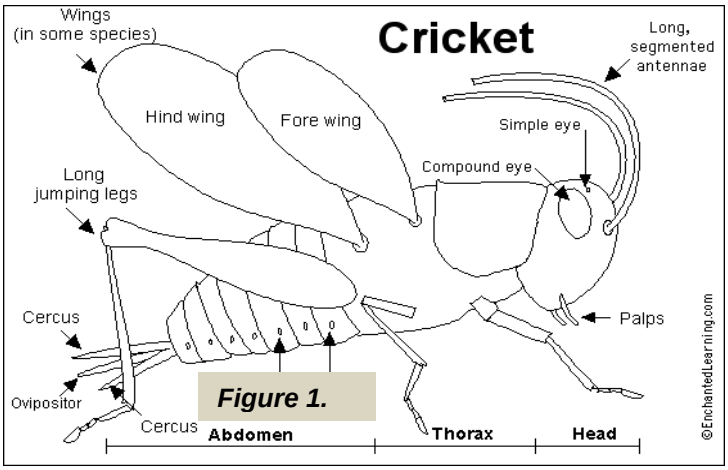


Figure 1.

Figure 2.

Crickets were used as a model to represent invertebrates typically caught in wet pit traps. Also, crickets were readily available and in the desired size/age range (The Reptile and Grow Store, Unit 7/117-119, Dixon Road, Rockingham, WA 6168; 95272215). They were kept in enclosed plastic boxes, under laboratory conditions of lighting and fed on a diet of carrots.

Visible morphological characteristics (see Figure 1.) which enable an assessment of the standard of preservation include but not limited to; the antennae, palps, legs, wings, ovipositor, head, thorax and abdomen. Internal organs were not examined.

The condition of specimens were also determined according to their recovery from ‘basic handling procedures’ for curation. It includes; pinning through the right side of the thorax for mounting (Figure 2), limb flexibility and positioning, brittleness, odour, oedema of abdomen, dystrophy of body parts or signs of disfigurement. Fine dissecting forceps were used to manouver body parts into desired positions.

DNA Preservation:

Preservation of genetic material suitable for molecular examination.

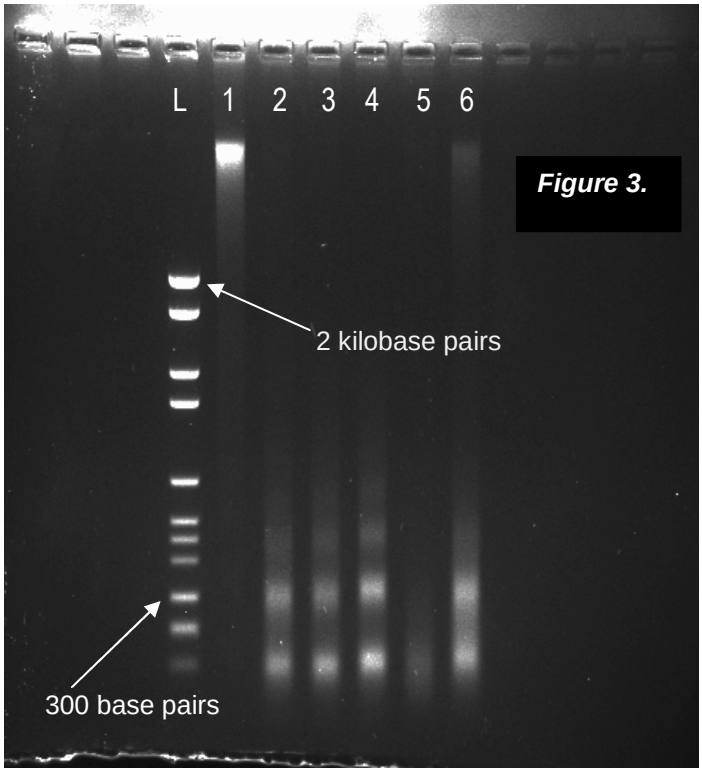


Figure 3.

The type of molecular examination that can be undertaken is limited by the size (and quality) of the isolated DNA. It can range from the cloning of large DNA fragments (more than 2 kilobase pairs) to the detection of single nucleotide polymorphisms (less than 200 base pairs). For example, the high quality DNA isolated from a freshly euthanized (by cryopreservation) cricket (Fig. 3, Lane 1) would be highly regarded for the range of molecular analyses available in a well resourced laboratory. However, the scope for analysis depends on the quality of DNA isolated. For example "preserving" crickets in sterile water alone will, after 7 days, result in extensively degraded DNA (Fig 3, Lane 2). The same is observed in the presence of pyroneg, a common laboratory detergent (Fig. 3, Lanes 3 – 6).

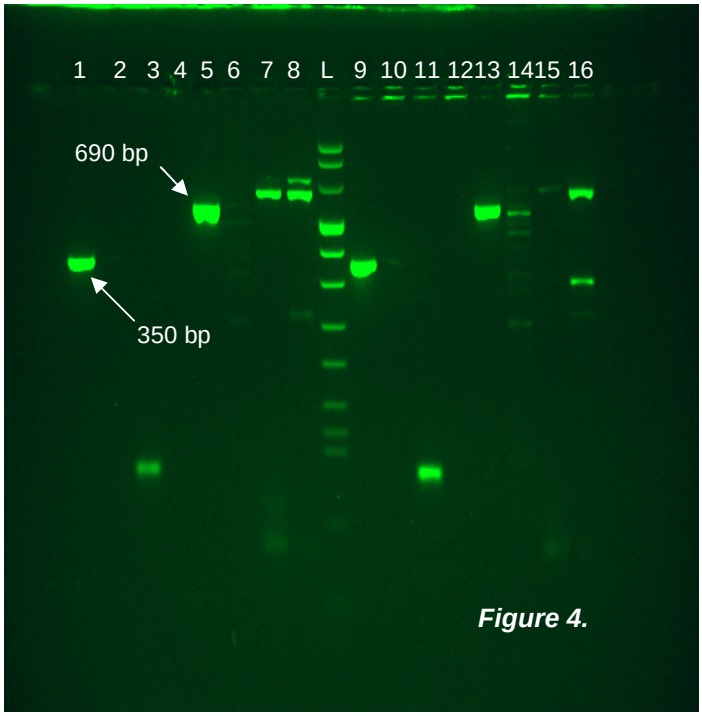


Figure 4.

Despite the apparent poorer than expected quality, degraded DNA (300 base pairs) can yield PCR-amplifiable products because of the robustness in PCR technology. The detection of amplified DNA of expected size is an indication that the DNA isolated is amenable to molecular examination. Of direct relevance to this project is the PCR amplification of conserved sequences in invertebrates, applicable to the cricket model. PCR products can be generated from genomic DNA of crickets. It includes the 350 bp and 690 bp fragments of mitochondrial DNA (Fig. 4, Lanes 1 and 5) and a 700 bp fragment from the invertebrate ribosomal internal transcribed region (Fig. 4 Lanes 7). Any one of these PCR products may be used as an extra quality control measure on DNA quality.

Preservative Stability

Solution stable for extended periods under extreme climatic conditions (eg. exposed to elements in Pilbara or Kimberly summer for up to three months)

To date, the science of wet pit trapping is largely based on short-term (up to one month) set up, capture and curation outcomes. A different approach is required for longer-term (2 to 3 months) trapping activity and especially under extreme conditions of temperature and wind velocity. It is highly desirable that the preservative/chemical solution arrangements; [1] are resistant to evaporation; [2] retain the killing and preserving capacities and; [3] in terms of handling and storage, do not pose a risk to humans and the environment.

[1] Evaporation. The suitability of ethanol for wet pit trapping is inherent in its chemical properties. Of immediate concern is the risk of loss from evaporation at high temperatures and wind velocities. Ethanol is an efficient preservative but compared to water and propylene glycol, it has a boiling point of 78.5^{0}C and a vapour pressure of 67.5 mm of Hg (Table 3). Its adoption is problematic unless a system is devised which minimizes evaporation.

Table 3: Physical and chemical properties

Preservative	Density	Boiling point	Vapour pressure	Other properties
Propylene glycol	1.036	188^{0}C	0.129 mm @ 25C)	Highly referenced, antifreeze, hygroscopic, unknown effects on DNA quality, non volatile
Ethanol	0.789	78.5^{0}C	67.5 mm @ 20C)	Highly referenced, especially on DNA preservation, evaporates readily, flammable, volatile
Water	1.00	100^{0}C	17.5 mm @ 20C)	No preservative properties

An 'overlay' could resolve the problem but provided that it; [1] is less dense than ethanol; [2] is stable for the life of the trapping program; [3] is resistant to evaporation; [4] enables ethanol to be prepared to the highest permissible starting concentration; [5] protects ethanol from evaporation; [6] safe to humans and to the environment and; [7] does not interfere with the killing/preserving process. In the laboratory, up to 25% of ethanol (70%) by weight can be lost to the environment through evaporation within 30 days in a 37^{0}C water-bath (Fig. 5C, blue dots). The rate of loss is lower at lower temperatures (Fig. 5A and 5B) but the 'protective effect' of a light mineral oil overlay (red dots) is clearly evident to account for the extreme volatility of ethanol.

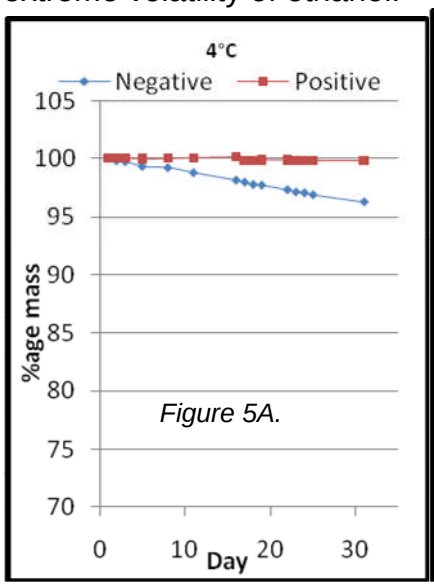


Figure 5A.

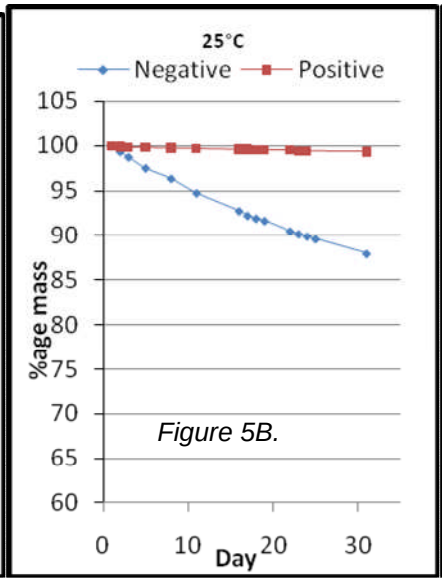


Figure 5B.

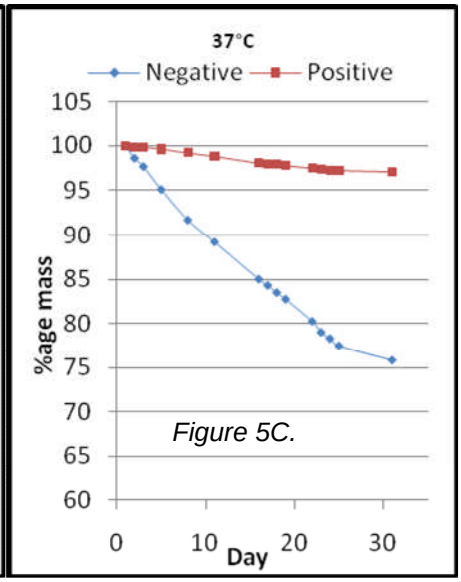


Figure 5C.

Mineral oil (also marketed under various names) is commonly used in the laboratory as overlay to prevent evaporation. Before the introduction of automation, PCR amplification relied exclusively on oil overlays as reaction mixtures then were subjected to repetitive cycles at extreme temperatures (eg. 35 cycles at 50^0C, 70^0C and 95^0C).

Table 4. List of Solution Overlays

	CAS Number	Molecular Formula	Molar mass	Density	Melting Point	Boiling Point	Solubility	Viscosity	Hazard
Mineral Oil (heavy)				0.862					
Mineral Oil (light)				0.838					
Mineral Oil (nujol)				0.880					
Mineral Oil (pure)	8042-47-5			0.877					
Mineral Oil (spectroscopy)	8042-47-5			0.851					
Mineral Oil (Sigma)	8042-47-5			0.840					
Olive Oil				0.703					
Ethanol	64-17-5	C2H5OH	46.07	0.789	-114.3	78.4	miscible	1.200 cP	F, Xn
Propylene glycol	57-55-6	C3H8O2	76.09	1.036	-59	188.2	miscible		
Paraffin Solution	8012-95-1			0.890	-18				
Water				1.000		100			

As can be seen in Table 4, mineral oil (0.838 – 0.877) is denser than ethanol (0.789). For mineral oil to assume the role of an overlay, ethanol would need to be diluted with water so that its density is more than that of mineral oil. Preliminary trials show that mineral oil will readily form an overlay over 80% ethanol which is an improvement over paraffin oil (Table 5).

Table 5: Comparison between Different Grades of Mineral Oil

	Ethanol Concentration					
Mineral Oil	0%	60%	70%	80%	90%	100%
[1] Pure grade	+	+	+	-	-	-
[2] Spectroscopy grade	+	+	+	+	-	-
[3] Sigma grade	+	+	+	+	-	-
[4] Paraffin Oil (control)	+	+	+	-	-	-

+ point of equivalence, mineral oil assumes overlay

The pure grade and paraffin oil versions required ethanol concentration to be further diluted to 70% with water. (Table 5). Both the spectroscopy grade and the 'Sigma grade' mineral oils enabled ethanol to be prepared to the highest permissible starting concentration (80%).

Another critical consideration was whether or not the overlay arrangement ensured rapid death for target (invertebrates) and non-target (vertebrates) species falling into the wet pit solution. Healthy crickets (*Figure 5;* adult or juvenile) dropping into 80% ethanol submerge quickly and are rapidly killed. The presence of a mineral oil overlay does not significantly alter the killing process except that juvenile crickets (25 mg; mean; *n* = 20) are prevented from entering the ethanol phase. The same was observed when it was substituted with euthanized adult cricket. With the current arrangements and using cricket as a model, target specimens that are less than 350 mg (mean; *n* = 20) are unlikely to overcome the overlay/fixative interface and consequently be held at that boundary.

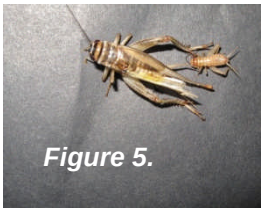


Figure 5.

Some surfactants were used as an attempt to overcome the effects of surface tension. They included [1] sodium docecyl sulphate (SDS; anionic); [2] cetyltrimethyl ammonium bromide (CTAB; cationic) and; [3] Triton X-100 (non-ionic/neutral). Ethanol (75%) was prepared with

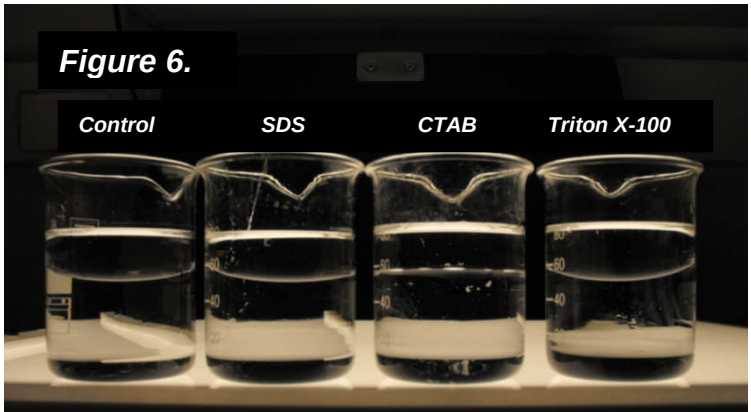


Figure 6.

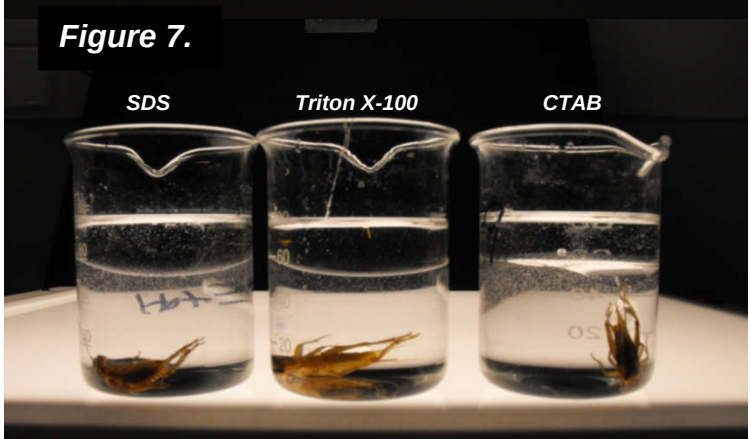


Figure 7.

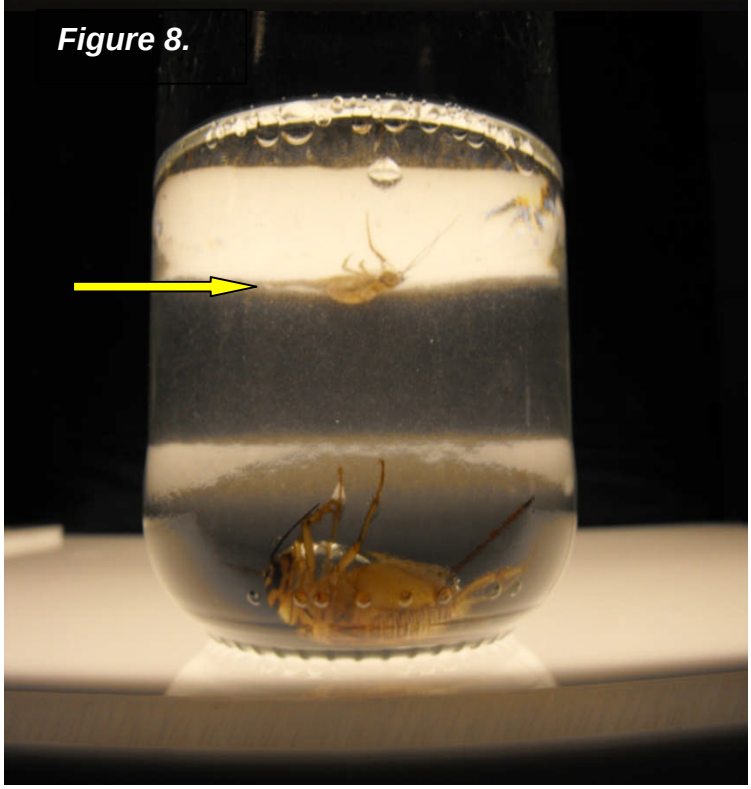


Figure 8.

or without 5% of SDS, CTAB or Triton X-100 and then overlayed with the same volume of mineral oil. The meniscus of each overlay was concave downwards (as in "without surfactant") except for the cationic CTAB/Mineral Oil arrangement which was slightly concave upwards (Fig. 6).

When a healthy adult cricket (350 mg) was dropped into a 'wet pit' trap of overlay/ethanol (non-surfactant control) it struggled briefly within the overlay. It then became disoriented but 'kicked or swam' strongly through the boundary layer and became submerged in the ethanol fixative. It rapidly died/drowned and then descended quickly to the bottom of the trap (Fig. 7).

As can be seen in Fig. 7, these observations were replicated in 'traps' containing sodium docecyl sulphate (anionic), Triton X-100 (non-ionic or neutral) and cetyltrimethyl ammonium bromide (cationic).

When the trial was repeated with a healthy juvenile cricket (25 mg), it struggled briefly within the overlay of the non-surfactant control. However, it was unable to break through to the ethanol fixative. Rather, it rapidly died/drowned in the overlay and then descended onto and remained on the overlay/ethanol interface (Fig. 8; yellow arrow).

There was no effect (reduction of surface tension) when ethanol was fortified with the surfactants sodium docecyl sulphate (anionic), Triton X-100 (non-ionic/neutral) or cetyltrimethyl ammonium bromide (cationic).

A different approach is required for longer-term (2 to 3 months) trapping activity and especially under extreme conditions. Statistics from the Australian Bureau of Meteorology show that surface temperatures over the summer months (November to February) can from 9 am to 3 pm, range from 27^{0}C to 37^{0}C (Tables 6A & 6B).

Table 6A: Mean 9am Conditions (Temperatures = C)

Location	J	F	M	A	M	J	J	A	S	O	N	D
Cue	29.4	28.3	25.8	21.5	16.3	12.7	11.6	13.5	17.1	20.7	25.0	28.1
Laverton	27.5	26.4	24.2	20.5	16.2	12.8	11.8	13.9	17.9	21.1	24.8	27.0
Leonora	27.7	26.2	24.1	20.4	15.5	11.8	10.9	13.0	17.0	20.9	24.0	26.5
Mt Magnet	29.0	27.9	25.5	20.8	16.1	12.6	11.6	13.1	16.8	20.6	24.3	27.3
Paynes Find	28.0	26.9	24.1	20.4	15.7	12.0	11,1	12.5	15.9	19.6	23.3	25.8
Yalgoo	28.0	27.1	24.6	20.4	15.6	12.4	11.0	12.6	16.1	19.4	23.5	26.6
Mean	**28.3**	**27.1**									**24.2**	**26.9**

Ref. Climate Statistics for Australian Locations (www.bom.gov.au) "Mean 9 am and 3 pm conditions"

Table 6B: Mean 3pm Conditions (Temperatures = C)

Location	J	F	M	A	M	J	J	A	S	O	N	D
Cue	36.5	35.5	33.0	28.0	22.4	18.5	17.7	19.7	23.8	27.4	31.7	35.0
Laverton	34.6	33.7	30.9	26.4	21.3	17.7	17.1	19.4	23.7	27.1	30.7	33.6
Leonora	35.8	34.2	31.8	26.9	21.8	18.2	17.6	19.7	24.0	27.8	31.0	34.1
Mt Magnet	36.8	35.4	33.2	27.8	22.6	18.5	17.9	19.7	23.9	27.8	31.6	35.1
Paynes Find	36.1	34.9	31.8	27.4	22.3	18.4	17.5	18.9	22.6	26.7	30.4	33.5
Yalgoo	36.0	35.2	32.5	27.5	22.2	18.4	17.4	19.1	23.0	26.4	31.0	34.4
Mean	**36.0**	**34.8**									**31.1**	**34.3**

Ref. Climate Statistics for Australian Locations (www.bom.gov.au) "Mean 9 am and 3 pm conditions"

An indication on the long-term effect(s) of ethanol and high temperatures on the structural integrity of specimens would greatly facilitate development of a wet pit arrangement which suits extreme conditions. Adult crickets were placed in a range of ethanol concentrations (60% to 90%) and incubated for 12 weeks at a range of temperatures (4^{0}C to 37^{0}C). Specimens were harvested at 2, 4, 8 and 12-week intervals, and their visible morphological charateristics compared with those of the control (untreated) and according to their recovery from 'basic handling procedures' of curation. All specimens were easily mounted regardless of ethanol concentration, temperature or duration of exposure (Fig. 9). Exposure to 90% ethanol at 37^{0}C, for 8 weeks, caused limbs to become inflexible (eg. hind legs) with a tendency to break. By the 12th week, appendages became brittle and extremely prone to breaking accidentally (eg. antenna). There were also signs of shrinkage and disfigurement.

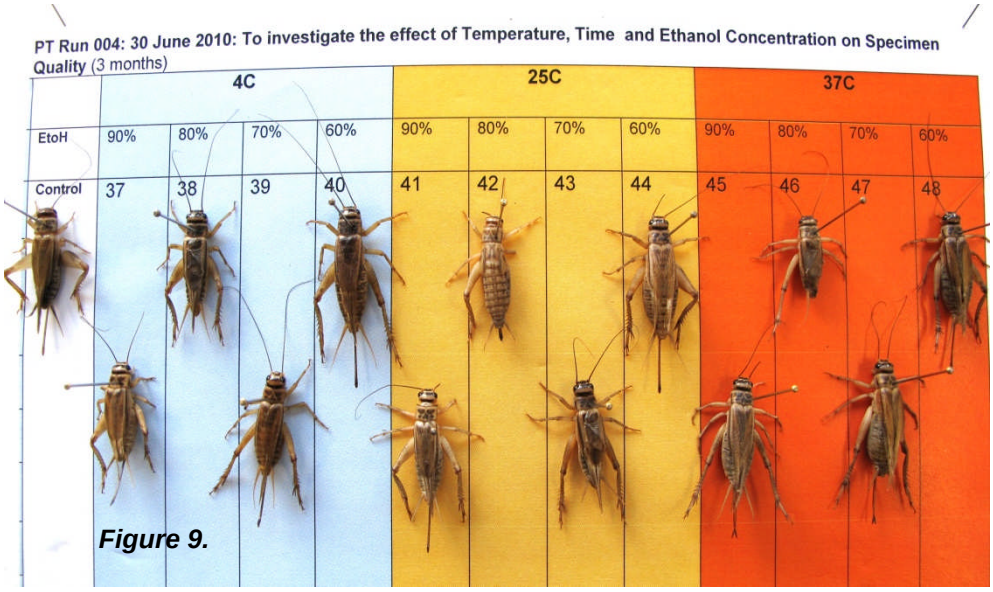
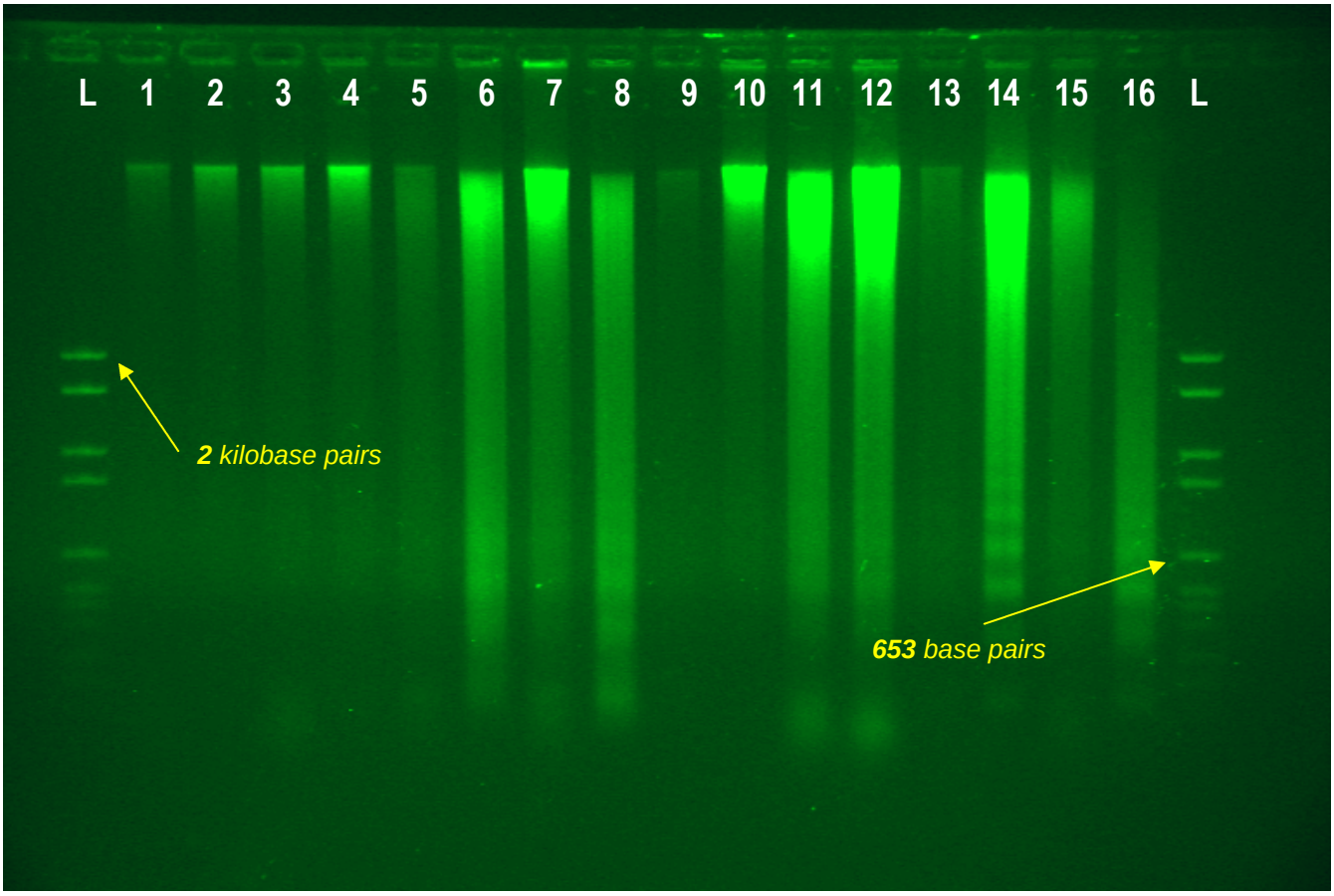


Figure 9.

The most preserved DNA resulted from using 90% ethanol as fixative, and was unaffected by temperature or duration of exposure (Fig. 10; 4^0C = Lanes 1 - 4 and; 37^0C = Lanes 9 -12). Clearly, prolonged exposure (12 weeks, 37^0C) of specimens to 60% ethanol resulted in a higher proportion of smaller DNA fragments (Fig. 10, Lane 16). Despite the extent of

Figure 10. Effect of Time and Temperature on DNA preservation by Ethanol



degradation, DNA isolates contained high molecular weight DNA (in excess of 2 kilobase pairs). Such DNA is generally regarded as suitable for molecular examination for example PCR amplification and DNA sequencing. Indeed, each isolate was positive for the 690 bp invertebrate mitochondrial gene by standard PCR amplification (Table 7). No adverse effects from ethanol were detected.

Table 7: Detection of 690 base pair Invertebrate Mitochondrial Gene by PCR amplification

		4C				25C				37C			
Ethanol (%)	C	90	80	70	60	90	80	70	60	90	80	70	60
Week 2	+	+	+	+	+	+	+	+	+	+	+	+	+
Week 4	+	+	+	+	+	+	+	+	+	+	+	+	+
Week 8	+	+	+	+	+	+	+	+	+	+	+	+	+
Week 12	+	+	+	+	+	+	+	+	+	+	+	+	+

C = Control: Thawed adult cricket and untreated.

As with ethanol, the suitability of **PROPYLENE GLYCOL** for wet pit trapping is inherent in its chemical properties (Table 2). Significantly, it is the safe alternative to ethylene glycol. Compared to water and ethanol, propylene glycol has a high boiling point (188.2^{0}C) and an extremely low vapour pressure (0.13 mm Hg, at 25^{0}C). Such features make it resistant to evaporation and an attractive proposition for long term wet pit trapping under extreme conditions given that, an overlay is no longer required. In the laboratory, it can be shown that both distilled water and ethanol (being volatile) are rapidly lost to the environment especially when the temperature is held at 45^{0}C (Table 8). By the 6th day, 25% of distilled water was lost to the atmosphere and up to 45% of ethanol had evaporated. However, propylene glycol gained weight but only marginally, from being hygroscopic.

Table 8. Effect of Temperature on the Rate of Evaporation

Day	Distilled Water	70% Ethanol	100% Propylene Glycol
0	27.62	25.93	27.01
1	26.78	23.62	27.25
2	26.31	22.09	27.54
6	20.99	14.28	28.20
% Loss	**25%**	**45%**	**-1.2%**

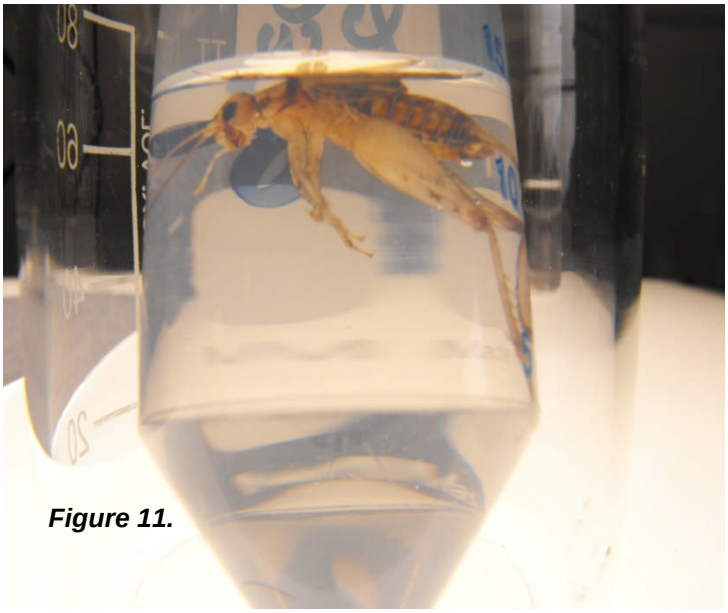


Figure 11.

A healthy adult cricket falling into propylene glycol (undiluted), is killed rapidly and becomes submerged immediately below the meniscus (see Fig. 11). The same is observed with juvenile cricket (5 mg) or an ant (0.8 mg) (data not shown). Over a period of 2 – 3 days, specimens (regardless of weight) descend further into the preservative. In contrast, the same specimens will submerge rapidly (within seconds) to the bottom of a container of ethanol (Fig. 12).

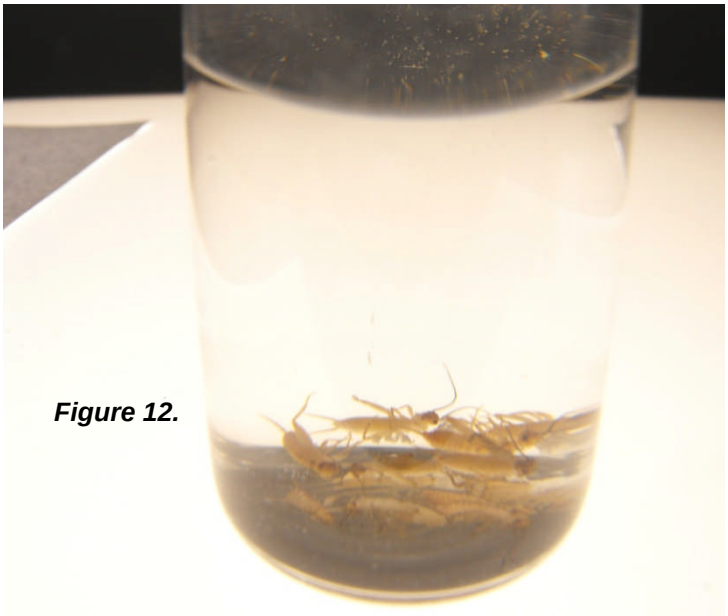


Figure 12.

Not much is known about the longer-term (more than one month) effects from exposure to propylene glycol at high temperatures. An adult cricket which had been fixed in propylene glycol for up to 8 weeks at 45^{0}C, was found to be adequately preserved (Fig. 13A, B & C). It was easily pinned onto the mounting board and the limbs retained their flexibility. There was no odour that indicated spoilage nor were there signs of shrinkage or disfigurement. Although the antennae were brittle, the majority of visual morphological characteristics were preserved. To investigate the effect of propylene glycol on DNA quality, juvenile crickets were placed in propylene glycol (undiluted) or in ethanol (75%) with

Figure 13A.

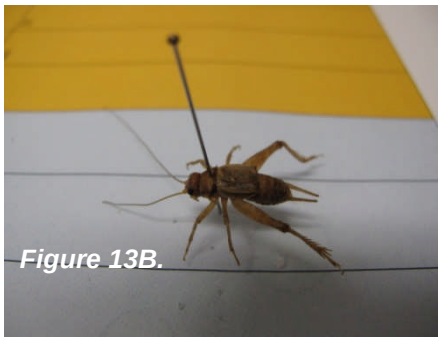


Figure 13B.

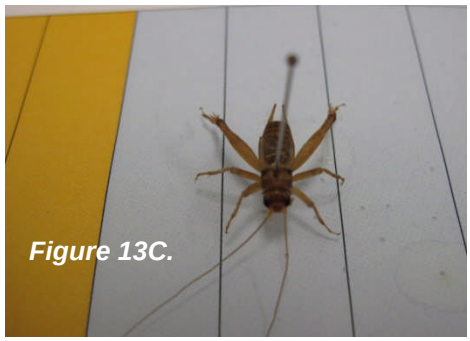


Figure 13C.

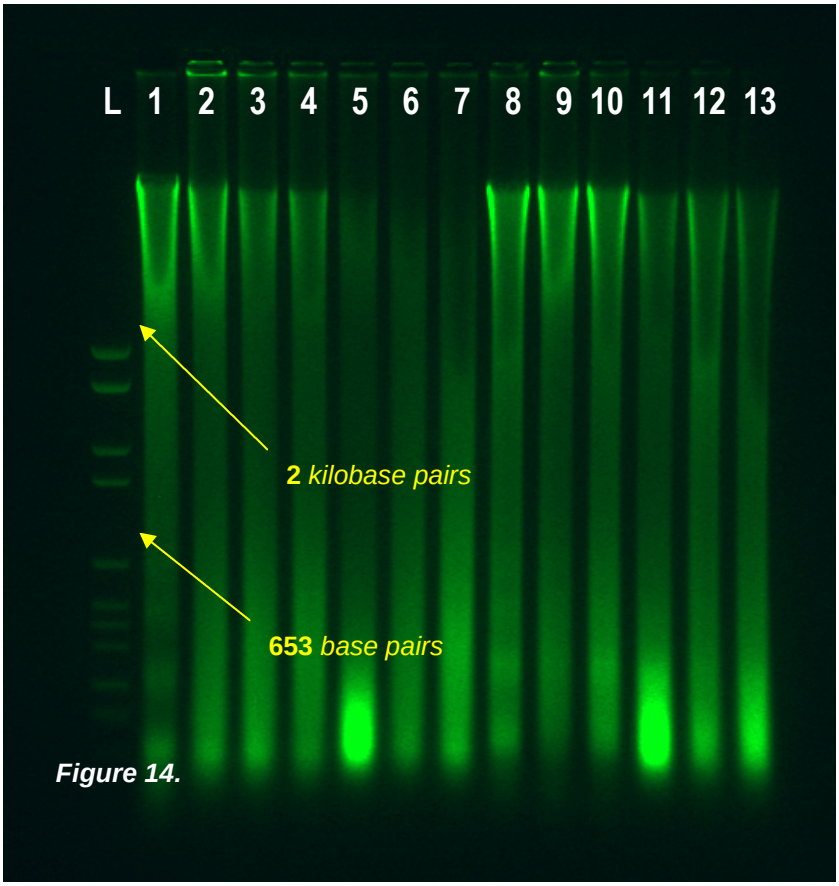


Figure 14.

mineral oil overlay, and incubated in a water bath for up to 6 weeks at 45^{0}C. Specimens were harvested at 1, 2, 3, 4 and 6-week intervals and DNA was isolated. The quality of DNA was assessed by electro-phoresis on agarose gel against a molecular weight ladder (Fig. 14; Lane L). Despite the degradation, high molecular weight DNA (more than 2 kilobases) was present in each of the isolates treated with either propylene glycol (Lanes 2 – 7) or 75% ethanol (Lanes 8 -13). Such DNA is generally regarded as suitable for molecular examination and PCR amplification (though not performed) is expected to generate the 690 bp fragment representing the invertebrate mitochondrial gene sequence. In this comparison, propylene glycol appeared to be as efficient as ethanol at preserving DNA.

Occupational Health and Safety

Minimal personal protective equipment required for safe use (ie. face shield in ventilated environment with gloves) and able to handle solution, or specimens preserved in solution, without health risk.

Propylene glycol, ethanol and mineral oil (light) are common laboratory chemicals. Their material safety data sheets (MSDS) are freely available from suppliers (see attachments). There is information about hazards, physical and chemical properties, and guidelines on first aid measures, fire fighting measures, handling and storage, exposure controls, personal protection, toxicology, ecotoxicity and disposal. The chemicals are generally non-hazardous in normal handling, except for ethanol which has higher material safety concerns (Table 9).

Table 9: Material Hazards Ranking

Chemical Name	CAS No.	Hazards Identification	NFPA Rating		
			Health	Flammability	Reactivity
Ethylene glycol	107-21-1	Adverse reproductive and fetal effects. Banned from use.	1	1	0
Propylene glycol	57-55-6	Non hazardous in normal handling, however good laboratory practices should always be used. Avoid long term exposure to skin or by inhalation.	0	1	0
Ethanol	64-17-5	Adverse reproductive and fetal effects. Flammable liquid and vapour. Good laboratory practices should always be used. Avoid long term exposure to skin or by inhalation.	2	3	0
Light Mineral Oil	8012-95-1	Generally not hazardous in normal handling, however good laboratory practices should always be used. Avoid long term exposure to skin or by inhalation.	1	0	0

National fire Protection Association (NFPA) Hazard Rating: 0 = least; 1 = slight; 2 = moderate; 3 = high; 4 = extreme

Conclusion

A summary of results is shown in Table 10 with the main focus on ethanol and related overlay arrangements. Ethanol-preserved specimens were sufficiently fixed and flexible, to be positioned and pinned onto the mounting board. The preservative was effective in the 60% to 80% concentration range, regardless of temperature (highest 37^0C) and for up to 12 weeks. The concentration of ethanol can be raised (90%) for molecular analyses though specimens tended to be brittle to handling. However, the ethanol concentration must be further diluted to enable the establishment of an overlay. Specific gene sequences were PCR-amplifiable in DNA isolated from the specimens despite the extent of DNA degradation.

Table 10:

Outcomes	Ethanol	Propylene Glycol
Animal Ethics	Kills rapidly, at 60% - 90%	Kills moderately rapidly, undiluted
Specimen Preservation	Visible morphological characteristics preserved at 60% - 80% for up to 12 weeks at 37^0C	Visible morphological characteristics preserved undiluted for up to 8 weeks at 45^0C
DNA Preservation	Amenability to molecular analysis preserved at 60% - 90% for up to 12 weeks at 37^0C	Potential for molecular analysis preserved undiluted for up to 6 weeks at 45^0C (as with ethanol)
Preservative Stability	Main concern is evaporation at extreme temperatures and wind velocities. Can adopt an 'overlay' arrangement though surface tension issues need to be resolved to enable all specimens to come in contact with preservative	No concerns with evaporation at extreme temperatures or wind velocities. No requirement for an overlay arrangement. All specimens come in contact with preservative
Occupational Health and Safety	Modest health hazard on good laboratory practice, highly flammable	Not a health hazard, low flammability

On a smaller scale, and as a comparison, propylene glycol was evaluated from advice from entomologists (DAFWA and associates). Limited trials showed that it could be a suitable substitute for ethanol given its unique physical and chemical properties. There was no requirement for an overlay and its hygroscopic nature would not be an issue in extremes of temp ıol on preservation of DNA.

Attachment 1

Material Safety Data Sheet **Ethanol, Absolute**

ACC# 89308

Section 1 - Chemical Product and Company Identification

MSDS Name: Ethanol, Absolute
Catalog Numbers: NC9602322
Synonyms: Ethyl Alcohol; Ethyl Alcohol Anhydrous; Ethyl Hydrate; Ethyl Hydroxide; Fermentation Alcohol; Grain Alcohol; Methylcarbinol; Molasses Alcohol; Spirits of Wine.
Company Identification:

Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410

For information, call: 201-796-7100
Emergency Number: 201-796-7100
For CHEMTREC assistance, call: 800-424-9300
For International CHEMTREC assistance, call: 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
64-17-5	Ethanol	ca.100	200-578-6

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: colorless clear liquid. Flash Point: 16.6 deg C.
Warning! Causes severe eye irritation. **Flammable liquid and vapor.** Causes respiratory tract irritation. This substance has caused adverse reproductive and fetal effects in humans. May cause central nervous system depression. May cause liver, kidney and heart damage. Causes moderate skin irritation.
Target Organs: Kidneys, heart, central nervous system, liver.

Potential Health Effects

Eye: Causes severe eye irritation. May cause painful sensitization to light. May cause chemical conjunctivitis and corneal damage.
Skin: Causes moderate skin irritation. May cause cyanosis of the extremities.
Ingestion: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. May cause systemic toxicity with acidosis. May cause central nervous system depression, characterized by excitement, followed by headache, dizziness, drowsiness, and nausea. Advanced stages may cause collapse, unconsciousness, coma and possible death due to respiratory failure.
Inhalation: Inhalation of high concentrations may cause central nervous system effects characterized by nausea, headache, dizziness, unconsciousness and coma. Causes respiratory tract irritation. May cause narcotic effects in high concentration. Vapors may cause dizziness or suffocation.
Chronic: May cause reproductive and fetal effects. Laboratory experiments have resulted in mutagenic effects. Animal studies have reported the development of tumors. Prolonged exposure may cause liver, kidney, and heart damage.

Section 4 - First Aid Measures

Eyes: Get medical aid. Gently lift eyelids and flush continuously with wate r.
Skin: Get medical aid. Wash clothing before reuse. Flush skin with plenty of soap and water.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.
Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get

medical aid. Do NOT use mouth-to-mouth resuscitation.
Notes to Physician: Treat symptomatically and supportively. Persons with skin or eye disorders or liver, kidney, chronic respiratory diseases, or central and peripheral nervous sytem diseases may be at increased risk from exposure to this substance.
Antidote: None reported.

Section 5 - Fire Fighting Measures

General Information: Containers can build up pressure if exposed to heat and/or fire. As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Vapors may form an explosive mixture with air. Vapors can travel to a source of ignition and flash back. Will burn if involved in a fire. Flammable Liquid. Can release vapors that form explosive mixtures at temperatures above the flashpoint. Use water spray to keep fire-exposed containers cool. Containers may explode in the heat of a fire.
Extinguishing Media: For small fires, use dry chemical, carbon dioxide, water spray or alcohol-resistant foam. For large fires, use water spray, fog, or alcohol-resistant foam. Use water spray to cool fire-exposed containers. Water may be ineffective. Do NOT use straight streams of water.
Flash Point: 16.6 deg C (61.88 deg F)
Autoignition Temperature: 363 deg C (685.40 deg F)
Explosion Limits, Lower:3.3 vol %
Upper: 19.0 vol %
NFPA Rating: (estimated) Health: 2; Flammability: 3; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Remove all sources of ignition. Use a spark-proof tool. Provide ventilation. A vapor suppressing foam may be used to reduce vapors.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Use only in a well-ventilated area. Ground and bond containers when transferring material. Use spark-proof tools and explosion proof equipment. Avoid contact with eyes, skin, and clothing. Empty containers retain product residue, (liquid and/or vapor), and can be dangerous. Keep container tightly closed. Keep away from heat, sparks and flame. Avoid ingestion and inhalation. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose empty containers to heat, sparks or open flames.
Storage: Keep away from heat, sparks, and flame. Keep away from sources of ignition. Store in a tightly closed container. Keep from contact with oxidizing materials. Store in a cool, dry, well-ventilated area away from incompatible substances. Flammables-area. Do not store near perchlorates, peroxides, chromic acid or nitric acid.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Use explosion-proof ventilation equipment. Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible

exposure limits.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Ethanol	1000 ppm TWA	1000 ppm TWA; 1900 mg/m3 TWA 3300 ppm IDLH	1000 ppm TWA; 1900 mg/m3 TWA

OSHA Vacated PELs: Ethanol: 1000 ppm TWA; 1900 mg/m3 TWA
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant a respirator's use.

Section 9 - Physical and Chemical Properties

Physical State: Clear liquid
Appearance: colorless
Odor: Mild, rather pleasant, like wine or whis
pH: Not available.
Vapor Pressure: 59.3 mm Hg @ 20 deg C
Vapor Density: 1.59
Evaporation Rate: Not available.
Viscosity: 1.200 cP @ 20 deg C
Boiling Point: 78 deg C
Freezing/Melting Point:-114.1 deg C
Decomposition Temperature: Not available.
Solubility: Miscible.
Specific Gravity/Density:0.790 @ 20°C
Molecular Formula:C2H5OH
Molecular Weight:46.0414

Section 10 - Stability and Reactivity

Chemical Stability: Stable under normal temperatures and pressures.
Conditions to Avoid: Incompatible materials, ignition sources, excess heat, oxidizers.
Incompatibilities with Other Materials: Strong oxidizing agents, acids, alkali metals, ammonia, hydrazine, peroxides, sodium, acid anhydrides, calcium hypochlorite, chromyl chloride, nitrosyl perchlorate, bromine pentafluoride, perchloric acid, silver nitrate, mercuric nitrate, potassium-tert-butoxide, magnesium perchlorate, acid chlorides, platinum, uranium hexafluoride, silver oxide, iodine heptafluoride, acetyl bromide, disulfuryl difluoride, tetrachlorosilane + water, acetyl chloride, permanganic acid, ruthenium (VIII) oxide, uranyl perchlorate, potassium dioxide.
Hazardous Decomposition Products: Carbon monoxide, irritating and toxic fumes and gases, carbon dioxide.
Hazardous Polymerization: Will not occur.

Section 11 - Toxicological Information

RTECS#:
CAS# 64-17-5: KQ6300000
LD50/LC50:
CAS# 64-17-5:
Draize test, rabbit, eye: 500 mg Severe;
Draize test, rabbit, eye: 500 mg/24H Mild;
Draize test, rabbit, skin: 20 mg/24H Moderate;
Inhalation, mouse: LC50 = 39 gm/m3/4H;
Inhalation, rat: LC50 = 20000 ppm/10H;
Oral, mouse: LD50 = 3450 mg/kg;
Oral, rabbit: LD50 = 6300 mg/kg;
Oral, rat: LD50 = 7060 mg/kg;

Oral, rat: LD50 = 9000 mg/kg;<BR.

Carcinogenicity:
CAS# 64-17-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: Ethanol has been shown to produce fetotoxicity in the embryo or fetus of laboratory animals. Prenatal exposure to ethanol is associated with a distinct pattern of congenital malformations that have collecetively been termed the "fetal alcohol syndrome".
Teratogenicity: Oral, Human - woman: TDLo = 41 gm/kg (female 41 week(s) after conception) Effects on Newborn - Apgar score (human only) and Effects on Newborn - other neonatal measures or effects and Effects on Newborn - drug dependence.
Reproductive Effects: Intrauterine, Human - woman: TDLo = 200 mg/kg (female 5 day(s) pre-mating) Fertility - female fertility index (e.g. # females pregnant per # sperm positive females; # females pregnant per # females mated).
Neurotoxicity: No information available.
Mutagenicity: DNA Inhibition: Human, Lymphocyte = 220 mmol/L.; Cytogenetic Analysis: Human, Lymphocyte = 1160 gm/L.; Cytogenetic Analysis: Human, Fibroblast = 12000 ppm.; Cytogenetic Analysis: Human, Leukocyte = 1 pph/72H (Continuous).; Sister Chromatid Exchange: Human, Lymphocyte = 500 ppm/72H (Continuous).
Other Studies: Standard Draize Test(Skin, rabbit) = 20 mg/24H (Moderate) Standard Draize Test: Administration into the eye (rabbit) = 500 mg (Severe).

Section 12 - Ecological Information

Ecotoxicity: Fish: Rainbow trout: LC50 = 12900-15300 mg/L; 96 Hr; Flow-through @ 24-24.3°CFish: Rainbow trout: LC50 = 11200 mg/L; 24 Hr; Fingerling (Unspecified)Bacteria: Phytobacterium phosphoreum: EC50 = 34900 mg/L; 5-30 min; Microtox test When spilled on land it is apt to volatilize, biodegrade, and leach into the ground water, but no data on the rates of these processes could be found. Its fate in ground water is unknown. When released into water it will volatilize and probably biodegrade. It would not be expected to adsorb to sediment or bioconcentrate in fish.
Environmental: When released to the atmosphere it will photodegrade in hours (polluted urban atmosphere) to an estimated range of 4 to 6 days in less polluted areas. Rainout should be significant.
Physical: No information available.
Other: No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	Not reviewed.	No information available.
Hazard Class:		
UN Number:		
Packing Group:		

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 64-17-5 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs
None of the chemicals in this material have an RQ.
SARA Section 302 Extremely Hazardous Substances
None of the chemicals in this product have a TPQ.
SARA Codes
CAS # 64-17-5: acute, chronic, flammable.
Section 313 No chemicals are reportable under Section 313.
Clean Air Act:
This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.
Clean Water Act:
None of the chemicals in this product are listed as Hazardous Substances under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
CAS# 64-17-5 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.

California Prop 65
WARNING: This product contains Ethanol, a chemical known to the state of California to cause developmental reproductive toxicity.
California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives
Hazard Symbols:
F
Risk Phrases:
R 11 Highly flammable.

Safety Phrases:
S 16 Keep away from sources of ignition - No smoking.
S 33 Take precautionary measures against static discharges.
S 7 Keep container tightly closed.
S 9 Keep container in a well-ventilated place.

WGK (Water Danger/Protection)
CAS# 64-17-5: 0
Canada - DSL/NDSL
CAS# 64-17-5 is listed on Canada's DSL List.
Canada - WHMIS
This product has a WHMIS classification of B2, D2A.
Canadian Ingredient Disclosure List
CAS# 64-17-5 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 7/27/1999
Revision #4 Date: 3/18/2003

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

Attachment 2

Material Safety Data Sheet

Propylene glycol

ACC# 19870

Section 1 - Chemical Product and Company Identification

MSDS Name: Propylene glycol
Catalog Numbers: AC158720000, AC158720010, AC158720025, AC158720050, AC158720200, AC220870000, AC220870010, AC220870250, P355-1, P355-20, P355-200, P355-4, S80150-1
Synonyms: 1,2-Dihydroxypropane; Methylethylene glycol; Monopropylene glycol; 1,2-Propylene glycol; 1,2-Propanediol; 2-Hydroxypropanol.
Company Identification:

Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410

For information, call: 201-796-7100
Emergency Number: 201-796-7100
For CHEMTREC assistance, call: 800-424-9300
For International CHEMTREC assistance, call: 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
57-55-6	Propylene glycol	>99	200-338-0

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: colorless viscous liquid.
Caution! May cause eye, skin, and respiratory tract irritation. Hygroscopic (absorbs moisture from the air).
Target Organs: Central nervous system.

Potential Health Effects

Eye: May cause slight transient injury.
Skin: May be absorbed through damaged or abraded skin in harmful amounts. Allergic reactions have been reported. A single prolonged skin exposure is not likely to result in the material being absorbed in harmful amounts. Prolonged contact is essentially non-irritating to skin. Repeated exposures may cause problems. Negative results have consistently been obtained in guinea pigs studies for sensitization. 1,2-Propylene glycol is not considered an occupational skin sensitizer. (CHEMINFO)
Ingestion: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. Low hazard for usual industrial handling. May cause hemoglobinuric nephrosis. May cause changes in surface EEG.
Inhalation: Low hazard for usual industrial handling. Inhalation of a mist of this material may cause respiratory tract irritation. Material has a low vapor pressure at room temperature, so exposure to vapor is not likely.
Chronic: Exposure to large doses may cause central nervous system depression. Chronic ingestion may cause lactic acidosis and possible seizures. Exposures to propylene glycol having no adverse effects on the mother should have no effect on the fetus. Birth defects are unlikely. In animal studies, propylene glycol has been shown not to interfere with reproduction.

Section 4 - First Aid Measures

Eyes: In case of contact, immediately flush eyes with plenty of water for a t least 15 minutes. Get medical aid.
Skin: In case of contact, flush skin with

plenty of water. Remove contaminated clothing and shoes. Get medical aid if irritation develops and persists. Wash clothing before reuse.
Ingestion: If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical aid.
Inhalation: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.
Notes to Physician: Persons with impaired kidney function may be more susceptible to the effects of this substance. Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion.
Extinguishing Media: Use water spray, dry chemical, carbon dioxide, or alcohol-resistant foam.
Flash Point: 99 deg C (210.20 deg F)
Autoignition Temperature: 371 deg C (699.80 deg F)
Explosion Limits, Lower:2.6 vol %
Upper: 12.6 vol %
NFPA Rating: (estimated) Health: 0; Flammability: 1; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Clean up spills immediately, observing precautions in the Protective Equipment section. Provide ventilation.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Use with adequate ventilation. Avoid contact with eyes, skin, and clothing. Keep container tightly closed. Avoid ingestion and inhalation.
Storage: Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances. Store protected from moisture.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Propylene glycol	none listed	none listed	none listed

OSHA Vacated PELs: Propylene glycol: No OSHA Vacated PELs are listed for this chemical.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.

Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Section 9 - Physical and Chemical Properties

Physical State: Liquid
Appearance: colorless viscous
Odor: Odorless
pH: Not available.
Vapor Pressure: 0.08 mm Hg @ 20 deg C
Vapor Density: 2.62 (air=1)
Evaporation Rate: Not available.
Viscosity: 58.1 cps @ 20 deg C
Boiling Point: 187 deg C
Freezing/Melting Point:-60 deg C
Decomposition Temperature: Not available.
Solubility: Soluble.
Specific Gravity/Density:1.030 g/ml
Molecular Formula:C3H8O2
Molecular Weight:76.09

Section 10 - Stability and Reactivity

Chemical Stability: Stable under normal temperatures and pressures. Hygroscopic: absorbs moisture or water from the air.
Conditions to Avoid: Excess heat, moist air.
Incompatibilities with Other Materials: Strong oxidizing agents.
Hazardous Decomposition Products: Carbon monoxide, carbon dioxide, aldehydes.
Hazardous Polymerization: Will not occur.

Section 11 - Toxicological Information

RTECS#:
CAS# 57-55-6: TY2000000
LD50/LC50:
CAS# 57-55-6:
Draize test, rabbit, eye: 100 mg Mild;
Draize test, rabbit, eye: 500 mg/24H Mild;
Oral, mouse: LD50 = 22 gm/kg;
Oral, mouse: LD50 = 20300 mg/kg;
Oral, rabbit: LD50 = 18500 mg/kg;
Oral, rat: LD50 = 20 gm/kg;
Skin, rabbit: LD50 = 20800 mg/kg;
Skin, rabbit: LD50 = 20800 mg/kg;
.

Carcinogenicity:
CAS# 57-55-6: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No information found
Teratogenicity: An expert panel convened by the NTP's Center for the Evaluation of Risks to Human Reproduction concluded 2/13/03 that developmental and reproductive risks stemming from exposure to the chemicals propylene glycol and ethylene glycol are negligible.
Reproductive Effects: When propylene glycol was given at 30 percent in the diet, it affected reproduction in rates in rats. It has generally not affected fertility or reproduction, except at very high doses where effects could be attributed to nutritional deficiency.
Mutagenicity: DNA Inhibition: Subcutaneous, mouse = 8000 mg/kg.; Cytogenetic Analysis: Subcutaneous, mouse = 8000 mg/kg.; Cytogenetic Analysis: Hamster, Fibroblast = 32 gm/L.
Neurotoxicity: No information found
Other Studies:

Section 12 - Ecological Information

Ecotoxicity: Water flea Daphnia: EC50 > 10000 mg/L; 48 Hr; UnspecifiedBacteria: Phytobacterium phosphoreum: EC50 = 710 mg/L; 30 min; Microtox testFish: Goldfish: LC50 > 5000 mg/L; 24 Hr;

UnspecifiedFish: Guppy: LC50 > 1000 mg/L; 48 Hr; Unspecified If released to water, 1,2-propanediol is expected to degrade relatively rapidly via biodegradation. If released to soil, relatively rapid biodegradation should also occur. Significant leaching in soil can be predicted.
Environmental: If released to the atmosphere, it is degraded rapidly by reaction with photochemically produced hydroxyl radicals (typical half-life of 32 hr). Physical removal from air by rainfall is possible.
Physical: No information available.
Other: No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	Not Regulated	Not Regulated
Hazard Class:		
UN Number:		
Packing Group:		

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 57-55-6 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs
None of the chemicals in this material have an RQ.
SARA Section 302 Extremely Hazardous Substances
None of the chemicals in this product have a TPQ.
Section 313 No chemicals are reportable under Section 313.
Clean Air Act:
This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.
Clean Water Act:
None of the chemicals in this product are listed as Hazardous Substances under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
CAS# 57-55-6 can be found on the following state right to know lists: Pennsylvania, Minnesota.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:

Not available.

Risk Phrases:

Safety Phrases:

S 24/25 Avoid contact with skin and eyes.

WGK (Water Danger/Protection)

CAS# 57-55-6: 0

Canada - DSL/NDSL

CAS# 57-55-6 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of Not controlled..
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

Canadian Ingredient Disclosure List

CAS# 57-55-6 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 5/27/1999
Revision #5 Date: 2/11/2008

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

Attachment 3

Material Safety Data Sheet

Ethylene Glycol P.A.

ACC# 95630

Section 1 - Chemical Product and Company Identification

MSDS Name: Ethylene Glycol P.A.
Catalog Numbers: AC295530000, AC295530010, AC295530025
Synonyms: 1,2-Dihydroxyethane, 1,2-Ethanediol, Ethylene alcohol, Ethylene dihydrate.
Company Identification:

Acros Organics N.V.
One Reagent Lane
Fairlawn, NJ 07410

For information in North America, call: 800-ACROS-01
For information in Europe, call: 0032(0) 14575211
For emergencies in the US, call CHEMTREC: 800-424-9300
For emergencies outside the US, call: 0032(0) 14575299

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
107-21-1	Ethylene glycol	99	203-473-3

Hazard Symbols: XN
Risk Phrases: 22

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: clear. **Caution!** Hygroscopic. This substance has causedadverse reproductive and fetal effects in animals. May cause kidneydamage. May cause central nervous system effects. May cause eye andskin irritation. May cause respiratory and digestive tractirritation. May cause cardiac disturbances.
Target Organs: Kidneys, heart, central nervoussystem, liver.

Potential Health Effects

Eye: May cause moderate eye irritation.
Skin: May cause skin irritation. Low hazard for usual industrial handling.
Ingestion: May cause nausea and vomiting. Toxicity follows 3-stage progression. (1) involves central nervous system effects including paralysis of eye muscles, convulsions, and coma. Metabolic acidosis and cerebral swelling may also occur. (2) involves cardiopulmonary system with symptoms of hypertension, rapid heart beat, and possible cardiac failure. (3) involves severe kidney abnormalities including possible renal failure.
Inhalation: May cause respiratory tract irritation. Heated or misted substance may cause headache, irregular eye movements, and possible coma.
Chronic: May cause kidney injury.

Section 4 - First Aid Measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids. Get medical aid immediately. Do NOT allow victim to rub or keep eyes closed.
Skin: Flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists. Wash clothing before reuse.
Ingestion: If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.
Inhalation: Remove from exposure to fresh air immediately. If not breathing,

give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
Notes to Physician: Ethanol may inhibit methanol metabolism.

Section 5 - Firefighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear.
Extinguishing Media: In case of fire, use water, dry chemical, chemical foam, or alcohol-resistant foam. Water or foam may cause frothing. Use agent most appropriate to extinguish fire.
Autoignition Temperature: 410 deg C (770.00 deg F)
Flash Point: 111 deg C (231.80 deg F)
NFPA Rating: health-1; flammability-1; reactivity-0 Explosion Limits, Lower: 3.20 vol % Upper: 15.30 vol %

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material, (e.g., dry sand or earth), then place into a chemical waste container. Remove all sources of ignition. Provide ventilation.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Use with adequate ventilation. Ground and bond containers when transferring material. Avoid contact with skin and eyes. Avoid ingestion and inhalation.
Storage: Keep away from sources of ignition. Store in a cool, dry, well-ventilated area away from incompatible substances.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Ethylene glycol	aerosol: 100 mg/m3	no established RELs - see Appendix D	none listed

OSHA Vacated PELs: Ethylene glycol: No OSHA Vacated PELs are listed for this chemical.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: Follow the OSHA respirator regulations found in 29CFR 1910.134 or European Standard EN 149. Always use a NIOSH or European Standard EN 149 approved respirator when necessary.

Section 9 - Physical and Chemical Properties

Physical State: Liquid
Appearance: clear

Odor: odorless
pH: Not available.
Vapor Pressure: .05 mm Hg @ 20C
Vapor Density: 2.1 (air=1)
Evaporation Rate:
Viscosity: mPas 20 deg C
Boiling Point: 195760.00 deg C
Freezing/Melting Point:-13 deg C
Decomposition Temperature: Not available.
Solubility: soluble in water
Specific Gravity/Density:1.1200g/cm3
Molecular Formula:C2H6O2
Molecular Weight:

Section 10 - Stability and Reactivity

Chemical Stability: Stable at room temperature in closed containers under normal storage and handling conditions.
Conditions to Avoid: Incompatible materials, ignition sources, moisture, excess heat.
Incompatibilities with Other Materials: Chlorosulfonic acid, dimethyl terephthalate, oleum, phosphorus pentasulfide, silvered-copper wire, sodium hydroxide, sulfuric acid, titanium butoxide. Causes ignition at room temperature with chromium trioxide, potassium permanganate, and sodium peroxide. Causes ignition at 100C with ammonium dichromate, silver chlorate, sodium chloride, and uranyl nitrate.
Hazardous Decomposition Products: Carbon monoxide, irritating and toxic fumes and gases, carbon dioxide.
Hazardous Polymerization: Has not been reported.

Section 11 - Toxicological Information

RTECS#:
CAS# 107-21-1: KW2975000
LD50/LC50:
CAS# 107-21-1:
Inhalation, rat: LC50 =10876 mg/kg;
Oral, mouse: LD50 = 5500 mg/kg;
Oral, rat: LD50 = 4700 mg/kg;
Skin, rabbit: LD50 = 9530 mg/kg;<BR.

Carcinogenicity:
CAS# 107-21-1:
ACGIH: A4 - not classifiable as a human carcinogen
Epidemiology: No data available.
Teratogenicity: No data available.
Reproductive Effects: There has been adverse reproductive effects in animals.
Neurotoxicity: No data available.
Mutagenicity: No data available.
Other Studies: Please refer to RTECS KW2975000 for additional information.

Section 12 - Ecological Information

Ecotoxicity: Goldfish LD50= >5000mg/L/24Hr Guppies LC50= 493,000ppm/7D Shrimp (salt water) LC50= >100ppm/48Hr
Environmental Fate: On soil, substance may leach to groundwater and biodegrade rapidly. In water, substance readily biodegrades. In air, substance reacts with hydroxyl radicals (T1/2= 1 day). Substance is not expected to bioconcentrate in marine life.
Physical/Chemical: No information available.
Other: Please refer to the Handbook of Envirnmental Fate and Exposure Data for additional information.

Section 13 - Disposal Considerations

Dispose of in a manner consistent with federal, state, and local regulations.
RCRA D-Series Maximum Concentration ofContaminants: None listed.
RCRA D-Series Chronic Toxicity ReferenceLevels: None listed.
RCRA F-Series: None listed.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	IATA	RID/ADR	IMO	Canada TDG
Shipping Name:	No information available.	Not regulated as a hazardous material.	Not regulated as a hazardous material.	Not regulated as a hazardous material.	No information available.
Hazard Class:					
UN Number:					
Packing Group:					

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 107-21-1 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
SARA

Section 302 (RQ)
None of the chemicals in this material have an RQ.
Section 302 (TPQ)
None of the chemicals in this product have a TPQ.
SARA Codes
CAS # 107-21-1: acute, chronic.
Section 313
This material contains Ethylene glycol (CAS# 107-21-1, 99%),which is subject to the reporting requirements of Section 313 of SARA Title III and 40 CFR Part 373.
Clean Air Act:
CAS# 107-21-1 is listed as a hazardous air pollutant (HAP). This material does not contain any Class 1 Ozone depletors. This material does not contain any Class 2 Ozone depletors.
Clean Water Act:
None of the chemicals in this product are listed as Hazardous Substances under the CWA. None of the chemicals in this product are listed as Priority Pollutants under the CWA. None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
CAS# 107-21-1 can be found on the following state right to know lists: California, New Jersey, Florida, Pennsylvania, Minnesota, Massachusetts.
California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives
Hazard Symbols:
XN
Risk Phrases:
R 22 Harmful if swallowed.
Safety Phrases:
S 2 Keep out of reach of children.
WGK (Water Danger/Protection)
CAS# 107-21-1: 0
Canada
CAS# 107-21-1 is listed on Canada's DSL/NDSL List.
WHMIS: Not available.
CAS# 107-21-1 is not listed on Canada's Ingredient Disclosure List.
Exposure Limits
CAS# 107-21-1: OEL-AUSTRALIA:TWA 60 mg/m3;STEL 120 mg/m3 OEL-BELGIUM:STEL 50 ppm (127 mg/m3) OEL-DENMARK:STEL 50 ppm (130 mg/m3) OEL-DENMARK:TWA 10 mg/m3 OEL-FINLAND:TWA 10 mg/m3;STEL 20 mg/m3

OEL-FINLAN
D:TWA 50 ppm (125 mg/m3);STEL 75
ppm (190 mg/m3) OEL-FRANCE:STEL 50
p
pm (125 mg/m3) OEL-HUNGARY:STEL 50
mg/m3;Skin OEL-THE NETHERLANDS:TW
A 10 mg/m3 OEL-THE
NETHERLANDS:TWA 50 ppm (125 mg/m3)
OEL-RUSSIA:STE
L 5 mg/m3 OEL-SWEDEN:TWA 50 ppm
(130 mg/m3);STEL 75 ppm (19 mg/m3) O
EL-SWITZERLAND:TWA 1 mg/m3 OEL-
SWITZERLAND:TWA 50 ppm (125 mg/m3)
OE
L-UNITED KINGDOM:TWA 10 mg/m3 OEL-
UNITED KINGDOM:TWA 60 mg/m3;STEL
12
5 mg/m3 OEL IN BULGARIA, COLOMBIA,
JORDAN, KOREA check ACGIH TLV OEL
IN NEW ZEALAND, SINGAPORE, VIETNAM
check ACGI TLV

Section 16 - Additional Information

MSDS Creation Date: 12/08/1995
Revision #3 Date: 3/12/1998

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no way shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

Attachment 4

Material Safety Data Sheet

Science Stuff, Inc.
1104 Newport Ave
Austin, TX 78753

Light Mineral Oil

Phone (512) 837-6020
Chemtrec 800-424-9300
24 Hour Emergency Assistance

Section 1 Identification

Product Number:	C2133
Product Name:	Mineral Oil Light Laboratory Grade
Trade/Chemical Synonyms	
Formula:	N/A
RTECS:	NONE
C.A.S	CAS# 8012-95-1

Health:	1
Flammability	0
Reactivity	0

Hazard Rating:

Least Slight Moderate High Extreme
0 1 2 3 4

NA = Not Applicable NE = Not Established

Section 2 Component Mixture

Sara 313	Component	CAS Number	%	Dim	Exposure Limits:
	Mineral Oil Light	CAS# 8012-95-1	100%	V/V	OSHA TWA 5 mg(mist)/mƒ

Section 3 Hazard Identification (Also see section 11)

Generally not hazardous in normal handling, however good laboratory practices should always be used. Avoid long term exposure to skin or by inhalation.

Section 4 First Aid Measures

Generally not hazardous in normal handling, however good laboratory practices should always be used. Avoid long term exposure to skin or by inhalation.

FIRST AID: SKIN: Wash exposed area with soap and water. If irritation persists, seek medical attention.

EYES: Wash eyes with plenty of water for at least 15 minutes, lifting lids occasionally. Seek Medical Aid. INHALATION: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen

INGESTION: Give several glasses of milk or water. Vomiting may occur spontaneously, but it is not necessary to induce. Never give anything by mouth to an unconscious person.

Section 5 Fire Fighting Measures

Fire Extinguisher Type:	Water spray, Carbon dioxide, dry chemical, powder, foam.
Fire/Explosion Hazards:	Negligible fire hazard when exposed to heat or flame
Fire Fighting Procedure:	Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and clothing.

Section 6 Accidental Release Measures

Absorb spill with inert material, then place in a chemical waste container. Remove all sources of ignition. Dispose of in a manner consistent with federal, local law.

Section 7 Handling and Storage

Keep away from heat and flame. Do not get in eyes, on skin, on clothing. Use with adequate ventilation.

Section 8 Exposure Controls & Personal Protection

Respiratory Protection:NIOSH/MSHA-approved respirator

Ventilation: Mechanical: ☐ Local Exhaust: ☑

Hand Protection: Gloves to prevent exposure

Eye Protection: Splash Goggles

Other Protective Equipment: Wear appropriate clothing to prevent skin exposure

Section 9 Physical and Chemical Properties

Melting Point:	Information not available	Specific Gravity	0.85
Boiling Point:	360°C	Percent Volatile by Volume:	0
Vapor Pressure:	Information not available	Evaporation Rate:	Information not available
Vapor Density:	Information not available	Evaporation Standard:	
Solubility in Water:	Insoluble	Auto ignition Temperature:	Information not available
Appearance and Odor:	Clear, colorless, odorless liquid	Lower Flamm. Limit in Air:	Information not available
Flash Point:	229°C (420°F)	Upper Flamm. Limit in Air:	Information not available

Section 10 Stability and Reactivity Information

Stability: Stable Conditions to Avoid: Temperature extremes

Materials to Avoid:
Strong oxidizing agents

Hazardous Decomposition Products:
Oxides of carbon

Hazardous Polymerization:Will Not Occur

Condition to Avoid:None known

Section 11 Additional Information

Effects of exposure: Inhalation hazard low except when misted/heated, which may result in irritation. Chronic exposure has caused lipoid pneumonia, fibrosis and paraffinoma. Prolonged contact may cause various skin disorders.

DOT Classification: Not Regulated

DOT regulations may change from time to time. Please consult the most recent version of the relevant regulations.

Revision No:0.1 Date Entered: 9/1/2006 Approved by: WPF

yes	M7113	SELECT CODE, [F