

AD-A163 216

CHEMICAL CHARACTERIZATION OF COMPOUNDS RELEASED BY  
MARINE MAMMALS(U) NAVAL OCEAN SYSTEMS CENTER KAILUA HI  
M G CERUTI AUG 83 NOSC/TR-930

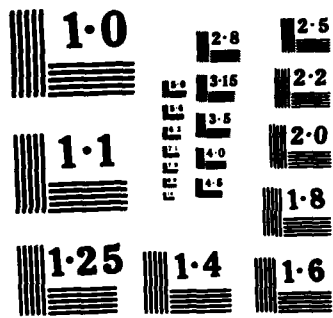
1/1

UNCLASSIFIED

F/G 6/1

NL

																					END ----	



NATIONAL BUREAU OF STANDARDS  
MICROCOPY RESOLUTION TEST CHART

4

NOSC TR 930

NOSC TR 930

Technical Report 930

# CHEMICAL CHARACTERIZATION OF COMPOUNDS RELEASED BY MARINE MAMMALS

AD-A163 216

DTIC  
ELECTE  
S JAN 22 1986 D  
D

MG Ceruti  
Code 512

December 1983

Prepared for  
Naval Sea Systems Command  
Code 6321

Approved for public release; distribution unlimited

DTIC FILE COPY

# NOSC

NAVAL OCEAN SYSTEMS CENTER  
San Diego, California 92152

86 2 24 034



NAVAL OCEAN SYSTEMS CENTER SAN DIEGO, CA 92152

---

AN ACTIVITY OF THE NAVAL MATERIAL COMMAND

J.M. PATTON, CAPT, USN

Commander

R.M. HILLYER

Technical Director

**ADMINISTRATIVE INFORMATION**

The work reported here was performed between January 1981 and March 1983 as a part of the Marine Animal Capabilities Program, sponsored by the Naval Sea Systems Command under Program Element 62759N.

This report summarizes documentation of some chemical analyses submitted by Dr PV Fennessey, Associate Professor of Pediatrics and Pharmacology and Susan S. Tjoa at the University of Colorado School of Medicine's Mass Spectrometry Research Resource, Denver, Colorado under contract numbers N66001-81-M-A236 and H66001-82-M-3175. Dr Fennessey and Ms Tjoa provided several enlightening discussions on results and analytical techniques.

The author extends gratitude to Dr PE Nachtigall and WA Friedl of Code 512 for their initiation, interest and support of this work; RW Hall, KV Keller and JL Richards of Code 512, and GA Peiterson for their assistance in sample collection; RH Brady of Code 446 for his editorial contributions; and K Wright, Code 4473, for her excellent work in literature searches.

Released by:  
PE NACHTIGALL, Head  
Research Division

Under authority of:  
HO PORTER, Head  
Biosciences Department

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM										
1. REPORT NUMBER NOSC Technical Report 930 (TR 930)	2. GOVT ACCESSION NO. AD-A163 216	3. RECIPIENT'S CATALOG NUMBER										
4. TITLE (and Subtitle) CHEMICAL CHARACTERIZATION OF COMPOUNDS RELEASED BY MARINE MAMMALS		5. TYPE OF REPORT & PERIOD COVERED Final: Jan 81 - May 83										
		6. PERFORMING ORG. REPORT NUMBER										
7. AUTHOR(s) MG Ceruti		8. CONTRACT OR GRANT NUMBER(s) N66001-81-M-236 N66001-82-M-3175										
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Ocean Systems Center, Hawaii Laboratory P.O. Box 997 Kailua, Hawaii 96734		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62759N; F59558; SF59558001; 512-MM02										
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Sea Systems Command, Code 6321 Department of the Navy Washington, D.C. 20361		12. REPORT DATE August 1983										
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		13. NUMBER OF PAGES 52										
		15. SECURITY CLASS. (of this report) Unclassified										
16. DISTRIBUTION STATEMENT (of this Report)  Approved for public release; distribution unlimited		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE										
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)												
18. SUPPLEMENTARY NOTES												
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)												
<table border="0"> <tr> <td>Chemoreception</td> <td>Toxicity</td> </tr> <tr> <td>Gas Chromatography</td> <td>Glandular Extracts</td> </tr> <tr> <td>Mass Spectrometry</td> <td>Perianal Gland</td> </tr> <tr> <td>Marine Mammal Excretions</td> <td>Prostate Gland</td> </tr> <tr> <td>Marine Mammal Secretions</td> <td></td> </tr> </table>			Chemoreception	Toxicity	Gas Chromatography	Glandular Extracts	Mass Spectrometry	Perianal Gland	Marine Mammal Excretions	Prostate Gland	Marine Mammal Secretions	
Chemoreception	Toxicity											
Gas Chromatography	Glandular Extracts											
Mass Spectrometry	Perianal Gland											
Marine Mammal Excretions	Prostate Gland											
Marine Mammal Secretions												
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)												
<p>Excretions, secretions and glandular extracts from marine mammals were analyzed chemically by gas chromatography and mass spectrometry to identify chemical constituents which may be involved in marine mammal chemoreception.</p> <p>The results of acidic, neutral and basic fractions of urine, feces, prostate gland extract and semen from male dolphins, <u>Tursiops truncatus</u>; of a urine sample from a female dolphin, <u>Tursiops truncatus</u>; and of one fecal sample from a male California sea lion, <u>Zalophus californianus</u>, are presented.</p>												

DD FORM 1473  
1 JAN 73EDITION OF 1 NOV 65 IS OBSOLETE  
S/N 0102-014-6601

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

The acidic and neutral fractions of a perianal gland secretion and fecal sample from the same male dolphin; and a fecal sample from a female Tursiops truncatus also are presented.

Various acids, esters, sugars, alcohols, steroids and nitrogen-containing compounds were in the samples. Taste and/or toxicity data were available for 25 compounds detected in the samples. Some sugars, some alcohols and glycine are not expected to be toxic.

Several compounds are noted as suitable stimuli for chemoreception experiments. These are lactic, phosphoric and succinic acids; glycine; urea; mannose; glycerol; inositol; arabitol; erythritol; mannitol; sorbitol; xylitol; erythrose; galactose; glucose; lactose; xylose; indole and skatole.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

## CONTENTS

INTRODUCTION . . .	7
CHEMICAL ANALYSIS . . .	9
MATERIALS AND METHODS . . .	9
ANALYSIS . . .	9
RESULTS . . .	10
DISCUSSION . . .	21
LIMITATIONS OF ANALYTICAL TECHNIQUES . . .	22
TASTE AND TOXICITY . . .	23
BACKGROUND . . .	23
RESULTS . . .	23
TASTE AND TOXICITY OF SOUR AND/OR BITTER SUBSTANCES . . .	23
Lactic Acid . . .	23
Succinic Acid . . .	28
Phosphoric Acid . . .	28
Urea . . .	28
Mannose . . .	28
TASTE AND TOXICITY OF GLYCINE AND ALCOHOLS . . .	28
Glycine . . .	28
Glycerol . . .	29
Inositol . . .	29
Arabitol . . .	29
Erythritol . . .	29
Mannitol . . .	29
Sorbitol . . .	29
Xylitol . . .	30
TASTE OF SWEET SUGARS . . .	30
General Comments . . .	30
Erythrose . . .	30
Galactose . . .	30
Glucose . . .	30
Lactose . . .	30
Mannose . . .	31
Xylose . . .	31
TOXICITY AND DISCUSSION OF COMPOUNDS WHICH ARE INSOLUBLE IN WATER AND/OR UNSAFE FOR CHEMORECEPTION EXPERIMENTS . . .	31
TOXICITY OF NITROGEN HETEROCYCLES . . .	31
RECOMMENDED EXPOSURE . . .	33
REFERENCES . . .	37
APPENDIX A: DISCUSSION OF SAMPLE COLLECTION TECHNIQUES . . .	51

## TABLES

1. Summary of substances detected by cetacea . . . 8
2. Principal chemical components in the acidic, neutral and basic fractions of a urine sample from a female Pacific bottlenose dolphin, Tursiops truncatus, (Tt-605) . . . 11
3. Principal chemical components in the acidic, neutral and basic fractions of a urine sample from a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-583) . . . 12
4. Principal chemical components in the acidic, neutral and basic fractions of four semen samples from a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-583) . . . 13
5. Principal components in the acidic, neutral and basic fractions of the extract of the prostate gland dissected from a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-026) . . . 14
6. Principal components in the acidic, neutral and basic fractions of blood from a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-026) . . . 15
7. Principal components in the acidic and neutral fractions of a perianal gland secretion of a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-042) . . . 16
8. Principal components in the acidic and neutral fractions of a fecal sample from a female Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-594) . . . 17
9. Principal components in the acidic and neutral fractions of a fecal sample from a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-042) . . . 18
10. Principal components in the acidic and neutral fractions of a fecal sample from a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-026) . . . 19
11. Principal chemical components in the acidic, neutral and basic fractions of a fecal sample from a male California sea lion, Zalophus californianus, (Zc-532) . . . 20



12. Summary of physical properties and toxicity information for sour and/or bitter compounds identified in the urine and semen sample of a male Atlantic bottlenose dolphin, Tursiops truncatus, (Tt-583) . . . 24
13. Summary of physical properties and toxicity information for one sweet amino acid and sweet polyhydric alcohols identified in excretions and secretions of marine mammals . . . 25
14. Summary of physical properties and toxicity information for sweet sugars identified in the acidic and neutral fractions of excretions and secretions of marine mammals . . . 26
15. Summary of physical properties and toxicity information for compounds insoluble in water and/or considered unsafe . . . 27
16. Recommended concentrations of compounds for use in chemoreception experiments . . . 34



Accession For		
NTIS	CRA&I	<input checked="" type="checkbox"/>
DTIC	TAB	<input type="checkbox"/>
Unannounced		<input type="checkbox"/>
Justification		
By		
Distribution/		
Availability Codes		
Dist	Availability/ or Special	
A-1		

## OBJECTIVE

Identify several chemical compounds found in the urine, perianal gland secretion, prostate gland, semen and/or feces of marine mammals which could be detected chemoreceptively. Provide information on the taste of appropriate chemicals and recommend safe levels of exposure for marine mammals in chemoreception experiments.

## RESULTS

1. The samples contained various acids, bases, steroids, polyhydric alcohols, phosphates, sugars, esters and neutral compounds. Not all constituents occurred in each sample.

2. All samples had constituents which could be used as stimuli in marine mammal taste reception studies.

3. Urine samples from a female Pacific bottlenose dolphin and a male Atlantic bottlenose dolphin had different constituent compounds.

4. Fecal samples from two male and one female Atlantic bottlenose dolphins also differed in chemical composition.

5. Some constituent compounds are not considered toxic. Safe concentrations of constituents which could be used in taste reception experiments can be estimated from laboratory studies on humans or animals or from concentrations used in foods or drugs prepared for human consumption.

6. Major components of marine mammal samples were similar to components from human controls.

7. High-level contamination from some plastic collection devices may have masked a large number of compounds present at low levels for some samples.

## RECOMMENDATIONS

1. For marine mammal chemoreception experiments, start with the following solution concentrations: 0.7% lactic acid, 0.05% phosphoric acid, 0.05 M urea, 0.02 M inositol, 2.0 M glycine, 2.8 M sorbitol, 1.0 M xylitol, 2.5 M galactose, 2.0 M glucose, 0.6 M lactose, 2.5 M xylose, 0.01% indole and 0.005% skatole.

2. Test glycerol, erythritol, arabitol, mannitol, mannose and erythrose for taste reception using solutions of 10%.

3. Avoid p-hydroxy-3-phenyllactic, 3,4-dihydroxybenzoic, 3,5-dihydroxybenzoic, palmitic, stearic, oleic, myristic and arachidic acids and cholesterol because they are inappropriate stimuli for chemoreception experiments due to toxicity or insolubility in water.

4. Use specially cleaned glass collection apparatus and techniques to allow analytic identification of low-concentration constituents.

## INTRODUCTION

Many terrestrial mammals use chemoreception, particularly olfaction, during feeding, territorial demarcation and reproduction (ref 1, 2). Marine mammals also may have some chemoreceptive capabilities (ref 3-5). Except for Soviet literature, chemoreception studies are mainly histological and anatomical (ref 6). Behavioral observations indicate that cetacea may be sensitive to chemical signals and the presence of blood (ref 3, 4).

Soviet studies tested a variety of chemicals that had a strong taste or smell, and porpoises and dolphins indicated detection either physiologically or behaviorally (ref 2, 3, 7-10). Evidence indicates that dolphins' chemoreception sensitivity to sugar, salt and some acids may be less than that of terrestrial mammals (ref 8).

Some compounds in marine mammal excretions and secretions may be chemoreceptively active (ref 3, 4, 7, 8). Table 1 summarizes the results of Kuznetsov's chemoreception experiments (ref 7-10). The harbor porpoise (Phocoena phocoena) can detect protein metabolites such as trimethylamine and skatole in seawater solutions at concentrations on the order of  $10^{-6}$  molar (ref 7, 10).

Soviet literature suggests that the perianal glands of male toothed whales may be involved in transmitting chemical signals directly into the water (ref 8).

In 1980, Naval Ocean Systems Center (NOSC) scientists began testing California sea lions' (Zalophus californianus) and bottlenose dolphins' (Tursiops truncatus) taste reception abilities. This study supports those tests by identifying possible chemoreceptively active compounds in marine mammal excretion and secretion samples.

PREVIOUS PAGE  
IS BLANK



SUBSTANCE	SOLVENT	CONCENTRATION	REF
Trimethylamine	Seawater	0.2%, 8.5 x 10 <sup>-6</sup> M	7,10
Trimethylamine	Seawater	0.2%	6
β-Phenylethyl			
Alcohol	Seawater	0.04% *	8
Indole	Seawater	0.01%	7,8
Indole	Fresh Water	10 <sup>-6</sup> M	10
Skatole	Seawater	1.7 x 10 <sup>-6</sup> M	10
Camphor	Seawater	0.01%	7,8
Camphor	Seawater	3 x 10 <sup>-6</sup> M	10
Quinine Chloride	Seawater	1 x 10 <sup>-4</sup> M	10
Quinine Chloride	Fresh Water	1 x 10 <sup>-5</sup> M	10
Hydrochloric			
Acid	Seawater	0.15 M	10
Hydrochloric			
Acid	Fresh Water	0.1 M	10
Caproic Acid	Fresh Water	5 x 10 <sup>-5</sup> M	10
Citric Acid	Seawater	0.2 M	10
Citric Acid	Fresh Water	0.05 M *	10
Oxalic Acid	Seawater	0.7 - 2.5% *	8
Oxalic Acid	Fresh Water	0.03 M	10
Picric Acid	Fresh Water	2 x 10 <sup>-5</sup> M *	10
Valeric Acid	Seawater	0.1 - 0.5% *	8
Valeric Acid	Fresh Water	1 x 10 <sup>-5</sup> M *	10
Valeric Acid	Seawater	1 x 10 <sup>-4</sup> M	10
Male Tursiops			
Urine	Seawater	Dilution of 10 <sup>-2</sup>	10

Table 1. Summary of substances detected by cetacea. Solvent indicates the carrier in which substances were dissolved. Concentrations are either a single value or a range over which chemoreceptive detection occurred. Threshold values are indicated by an asterisk.

## CHEMICAL ANALYSIS

### MATERIALS AND METHODS

One sample of urine and four of semen (sperm and/or seminal fluid) were obtained from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-583). One fecal sample from a male California sea lion Zalophus californianus (Zc-532) was collected in a plastic container. The dolphin samples were transferred immediately to separate glass vials for storage and shipping. One urine sample from a female dolphin, Tursiops truncatus (Tt-605), was collected in a plastic cup.

The following samples were collected in pre-cleaned sterilized glass jars or test tubes. During necropsy, the prostate gland was dissected from a male dolphin (Tt-026) and placed in a glass jar. Fecal and blood samples also were collected from the same animal during the dissection. A milky white perianal gland secretion was collected from a live male dolphin (Tt-042). Fecal samples also were obtained from two dolphins, Tt-594, a female, and Tt-042, a male.

All samples were frozen immediately. Between several days and three months later, they were sent to the University of Colorado Health Sciences Center for analysis by Dr PV Fennessey and Susan S. Tjoa. The samples were packed in dry ice during shipment.

### ANALYSIS

All except three samples were separated into three organic fractions (acidic, neutral or basic) by solvent extraction and analyzed by gas chromatography and mass spectrometry (ref 11, 12). Details of the technique are described in a report obtained under contract for the analyses (ref 13). Copies are available from the author upon request.

For the samples from Tt-583 and Zc-532, one to five ml of rinse water from an empty plastic bag, a plastic container, and an empty glass vial similar to those used in sample collection and shipment were analyzed in the gas chromatograph for background contamination. Malonic acid was the internal standard and C<sub>24</sub>H<sub>50</sub>, a hydrocarbon, was the external standard.

Substances were identified from retention time in the chromatograph and from charge-to-mass ratios in the spectrometer. Relative amounts of components were indicated by the area under the chromatographic curves (ref 11).

Four types of gas chromatographic columns were used to separate the components. Packed column types, OV-22 and OV-1, were used to analyze the samples from Tt-583 and Zc-522. Capillary gas columns, types SE-30 and OV-101, were used to analyze the acidic and neutral fractions of the other samples. The basic fractions of the urine from Tt-605 and of all samples from Tt-026 also were analyzed with the SE-30 column.

## RESULTS

The results from analyses of dolphin urine, semen, prostate glandular extract, blood, perianal gland secretion, feces and of sea lion feces are in tables 2-11. The amounts of perianal gland secretion from Tt-042 and feces from Tt-594 and Tt-042 were too small to permit analyses of the base fractions. Therefore, data for only the acidic and neutral fractions are shown in tables 7, 8 and 9 for those samples. All tables also indicate the estimated relative amounts of the various constituents. The chromatographic column type used to obtain the data also is indicated in the tables.

Some compounds, such as long chain fatty acids and/or lipids, sugars and polyhydric alcohols, were found in many samples: for example, palmitic acid (7 samples), stearic acid (10 samples) and oleic acids (5 samples).

Phosphoric acid was found in at least five samples. It was the most abundant component in the acidic fractions of (a) two semen samples from Tt-583, (b) the fecal sample from Tt-594 and (c) the fecal sample of Zc-532. Phosphoric acid also may have been in the neutral fraction of the perianal gland sample (table 7).

Polyhydric alcohols also were abundant in many samples. Except for one semen sample, inositol was found in every sample. Inositol was the most abundant component in the various fractions of other semen samples, prostate gland extracts and blood. Mannitol, another polyhydric alcohol, occurred in six samples. Mannitol was most abundant in neutral fractions of semen sample number one (table 4), the prostate gland extract (table 5) and dolphin feces (table 8).

Several sugars were detected. Glucose occurred in at least four samples. Erythrose was the most abundant neutral component in the fecal sample from Tt-026.

Some compounds were identified only once in these samples. Urine from Tt-583 had six acids and at least one neutral compound not found in any other sample. Similarly, the fecal sample from Zc-532 contained one acid, one sugar and three bases unique to the samples analyzed. Talose, the most abundant neutral component, is an example (table 11).

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE		NEUTRAL FRACTION		ESTIMATED RELATIVE ABUNDANCE		BASIC FRACTION		ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101	SE-30	OV-101	SE-30	OV-101	SE-30	OV-101	
Palmitic acid	0.042	1.0	Palmitic acid or a palmitate ester	0.33	0.91	Palmitic acid or a palmitate ester			1.0
Stearic acid	0.042	0.0	Stearic acid or a stearate ester	0.45	1.0	Stearic acid or a stearate ester			0.23
p-hydroxy-3-phenyl-lactic acid	0.0	1.0	Erythritol	0.0	0.080				
p-hydroxyphenyl-acetic acid	1.0	0.0	Inositol	1.0	0.98				
Threonic acid	0.94	0.0	Cholesterol	0.0	0.074				
A phosphate	0.0	0.35	3-β-hydroxy-5-stigmastene	0.0	0.086				

Table 2. Principal chemical components in the acidic neutral and basic fractions of a urine sample from a female Pacific bottlenose dolphin, Tursiops truncatus, (Tt-605). The estimated relative abundances are indicated with those of the most abundant component in each fraction, for each chromatographic column type, equal to one.



ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE	BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	OV-22		OV-1		OV-22
Succinic acid	0.4	Arabitol	0.13	Urea	1.01
Phosphoric acid	0.5	Ribitol	0.11		
Threonic acid	1.0	Inositol	0.02		
Erythronic acid	0.8	Erythrose	1.0		
Threitol	0.2	Mannose	0.09		
Erythritol	0.3	Galactose	0.06		
Lactic acid	0.4	Glucose	0.09		
p-Hydroxy-3-phenyllactic acid	0.3	Lactose	0.04		
$\beta$ -Hydroxybutyric acid	0.5	Glucosamine (?)			
$\beta$ -Hydroxyvaleric acid	0.5	Other sugars (?)			
2-Keto-3-methylbutyric acid	0.4				

Table 3. Principal chemical components in the acidic, neutral and basic fractions of a urine sample from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-583). The estimated relative abundances are indicated with those of the most abundant component equal to one. The chromatographic column type is indicated for each fraction. Question marks indicate tentative assignments.

ACIDIC FRACTION	OV-22			OV-1			OV-22				
	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE	BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	sample #	sample #	sample #	sample #		
	1	2	3		1	2	3	1	2	3	4
Stearic acid	0.01	0.03	0.5	Inositol	0.0	1.0	1.0	Urea	0.33	1.0	1.0
Oleic acid	0.0	0.03	0.0	Galactose	0.0	0.0	0.33	A palmitic acid or a palmitate ester			
Citric acid	0.12	0.0	0.0	Glucose	0.0	0.0	0.44		0.55	0.0	0.0
Glycine	0.03	0.0	0.0	Mannitol	1.0	0.0	0.0	Stearic and oleic acids or stearate and oleate esters			
Inositol	0.06	0.0	1.0								
Glycerol	0.22	0.0	0.0								
Phosphoric acid	1.0	1.0	0.5						1.0	0.0	0.0
$\alpha$ -Glycerophosphate		0.0	0.5								
$\beta$ -Glycerophosphate	0.7	0.0	0.33								
Cholesterol (?)											

Table 4. Principal chemical components in the acidic, neutral and basic fractions of four semen samples from a male Atlantic bottlenose dolphin, *Tursiops truncatus* (Tt-583). The estimated relative abundances are indicated with those of the most abundant component of each sample equal to one. The chromatographic column type is indicated for each fraction. Question mark indicates tentative assignment.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE		NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE		BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101		SE-30	OV-101		
Palmitic acid	0.081	0.0	Sorbitol	0.002	0.45	Palmitic acid or a palmitate ester	1.0
Stearic acid	0.11	0.0	Mannitol	0.29	0.48	Stearic acid or a stearate ester	0.97
Oleic acid	0.031	0.0	Ribitol	0.0	0.086	Oleic acid or an oleate acid	0.47
Citric acid	0.045	0.0	Arabitol	0.0	0.031	p-hydroxyphenyl-lacetic acid or a p-hydroxyphenylacetate ester	0.090
$\alpha$ -Glycerophosphate	0.15	0.0	Inositol	1.0	1.0	Phosphate	0.041
$\beta$ -Glycerophosphate	0.021	0.0	Palmitic acid and a sugar (mixture) or palmitate ester and a sugar (mixture)	0.038	0.0		
Inositol	0.040	1.0					
Mannose or glucose phosphate	0.042	0.0					
A sugar	1.0	0.0					
A sugar phosphate	0.85	0.0					

Table 5. Principal chemical components in the acidic, neutral and basic fractions of an extract from the prostate gland of a male Atlantic bottlenose dolphin, *Tursiops truncatus* (Tt-026). The estimated relative abundances are indicated with those of the most abundant component in each fraction for each chromatographic column type, equal to one.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE	BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101	SE-30	OV-101	SE-30
Palmitic acid	0.14	0.0	1.0	0.0	1.0
		Palmitic acid or a palmitate ester			Palmitic acid or a palmitate ester
Stearic acid	1.0	0.0	0.10	0.0	0.63
		Stearic acid or a stearate ester			Stearic acid or a stearate ester
Phosphoric acid	0.041	0.0	0.0	0.19	0.22
		Mannitol			Oleic acid or an oleate ester
Inositol	0.68	0.0	0.0	0.24	0.29
		Sorbitol			Myristic acid or a myristate ester
		Inositol		1.0	0.18
		Arabitol		0.046	0.056
					Phosphate
					Cholesterol

Table 6. Principal chemical components in the acidic neutral and basic fractions of a blood sample from a male Atlantic bottlenose dolphin, Tursiops truncatus (Tt-026). The estimated relative abundances are indicated with those of the most abundant component in each fraction, for each chromatographic column type, equal to one. In the acidic fraction, no compounds were detected with the OV-101 column.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30		SE-30 OV-101
Palmitic acid	0.89	Palmitic acid or a palmitate ester	0.0 0.86
Stearic acid	1.0	Stearic acid or a stearate ester	0.0 1.0
Oleic acid	0.13	Phosphoric acid or a phosphate	1.0 0.0
3,4-Dihydroxybenzoic acid	0.06		
3,5-Dihydroxybenzoic acid	0.04		
Isocitric acid	0.23		
Inositol	0.28		

Table 7. Principal chemical components in the acidic and neutral fractions of a perianal gland secretion sample obtained from a male Atlantic bottlenose dolphin *Tursiops truncatus* (Tt-042). The estimated relative abundances are indicated with those of the most abundant component in each fraction for each chromatographic column type equal to one.

ACIDIC FRACTION	ESTIMATED ABUNDANCE	NEUTRAL FRACTION	ESTIMATED ABUNDANCE
	SE-30		OV-101
Palmitic acid	0.74	0.0	0.0
Stearic acid	1.0	0.0	0.0
Oleic acid	0.11	0.0	0.0
Isocitric acid	0.22	0.0	0.0
p-Hydroxyphenylacetic acid	0.13	0.0	0.0
Phosphoric acid	0.0	1.0	0.0
		Stearic acid or a stearate ester	0.0
		Glucose	0.0
		Erythrose	0.30
		Mannitol	0.38(?)
		Inositol	0.39
		A sugar	0.0
		A sugar-phosphate	0.095
		Another sugar-phosphate	1.0

Table 8. Principal chemical components in the acidic and neutral fractions of a fecal sample from a female Atlantic bottlenose dolphin, Tursiops truncatus (Tt-594). The estimated relative abundances are indicated with those of the most abundant component of each column type, equal to one. Question mark indicates tentative assignment on the SE-30 column.

ACIDIC FRACTION	ESTIMATED ABUNDANCE	NEUTRAL FRACTION	ESTIMATED ABUNDANCE
	SE-30	OV-101	SE-30
		OV-101	OV-101
Palmitic acid	1.0	0.0	0.81
		Palmitic acid or a palmitate ester	0.0
Stearic acid	0.55	0.0	1.0
		Stearic acid or a stearate ester	1.0
Oleic acid	0.18	0.0	0.0
		Gluconolactone	0.49
Myristic acid	0.0	1.0	0.082
		Erythrose	0.0
		Erythritol	0.49
		Xylitol (?)	0.41
		Mannitol	0.61
		Inositol	0.13
			0.16

Table 9. Principal chemical components in the acidic and neutral fractions of a fecal sample obtained from a male Atlantic bottlenose dolphin, *Tursiops truncatus* (Tt-042). The estimated relative abundances are indicated with those of the most abundant component in each fraction, for each chromatographic column type, equal to one. Question mark indicates tentative assignment.

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE	BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30	OV-101	SE-30	OV-101	SE-30
Palmitic acid	0.67	1.0	0.27	0.24	1.0
Stearic acid	1.0	0.88	0.42	0.02	0.81
Myristic acid	0.0	0.25	0.0	0.02	
$\alpha$ -Glycerophosphate	0.15	0.0	1.0	1.0	0.11
Inositol	0.032	0.0	0.0	0.38	0.51
A phosphate	0.0	0.38	0.67	0.43	0.065
			0.014	0.0	

Table 10. Principal chemical components in the acidic, neutral and basic fractions of a fecal sample from a male Atlantic bottlenose dolphin, *Tursiops truncatus* (It-026). The estimated relative abundances are indicated with those of the most abundant component in each fraction, for each chromatographic column equal to one. Question mark indicates tentative assignment.



ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE	BASIC FRACTION	ESTIMATED RELATIVE ABUNDANCE
	OV-22		OV-1		OV-22
Phosphoric acid	1.0	Glucose	0.75	Glycerol	0.06
3-(4-Hydroxyphenyl) propionic acid	0.3	Galactose	0.05	Palmitic acid or a palmitate ester	1.0
		Talose	1.0	Arachidic acid or an arachidate ester	0.29
		Xylitol	0.1	Stearic and oleic acid or stearate and oleate esters	0.16
				Tyramine	0.59
				Tryptamine	0.27

Table 11. Principal chemical components in the acidic, neutral and basic fractions of a fecal sample from a male California sea lion, Zalophus californianus (Zc-532). The estimated relative abundances are indicated those of the most abundant component equal to one. The chromatographic column type also is indicated for each fraction.

At least seven other chemicals from five other samples were detected only once in this series.

Urea, the only component found in the basic fraction of the urine sample from the male dolphin (table 3), also occurred in the basic fraction of three semen samples.

## DISCUSSION

The majority of compounds detected in the acidic and basic fractions of the urine, semen and prostate gland samples also occur in samples from other mammals, including humans (ref 14-68). Citric acid and glycine are common in mammalian semen. Human semen also contains inositol (ref 43, 44, 49-52).

Glycerol was in the basic fraction of sea lion feces, and several long chain fatty acids occurred in the basic fractions from dolphin urine, semen, prostate gland extract, feces and blood. These acids may have been attached as esters to glycerophosphate amine compounds in the samples (ref 13).

Fatty acids also occurred in the neutral fractions of dolphin urine, prostate gland extract, feces and blood. These acids may have been present in their lipid forms (as esters) in the samples and thus would tend to a more neutral than acidic character in the analyses. A similar transformation could be true for the acid/esters mentioned above. Because such ester transformation is possible, the exact original compounds present in the neutral and basic fractions of some samples were indeterminable.

Several compounds in the acidic and neutral fractions of the blood sample (table 6) may be involved in cetaceans' purported chemoreceptive sensitivity to blood (ref 3, 4). These compounds are discussed below in the section entitled "Taste and Toxicity."

The chemical analysis of the perianal gland secretion (table 7) is the first for marine mammals (ref 69). The perianal gland secretion from the dolphin was milky white and possibly contained lipids (esters of fatty carboxylic acids) and carboxylic acids. Lipids have been reported in perianal gland secretion from the black-tailed prairie dog, Cynomys ludovicianus (ref 70), and carboxylic acids occur in anal scent pockets and glands of the Indian mongoose, Herpestes auropunctatus, and of the red fox, Vulpes vulpes, respectively (ref 1, 71). Moreover, green and white secretions from otter (Lutra lutra) anal scent sacs also contain lipids and fatty acids (ref 2).

## LIMITATIONS OF ANALYTICAL TECHNIQUES

Background contamination from the plastic bags and cup used in sample collection interfered mainly with neutral fraction analyses. Thus, the neutral fractions reported for samples Tt-583 and Zc-532 indicate only major components. Urine was collected from Tt-605 in a plastic cup, but analytic interference was less than from the plastic bags. All other samples were collected in specially cleaned glassware which was obtained from the analytical laboratory in Colorado. Samples collected in the glassware were free from container contamination. Sample collection techniques are discussed in appendix A.

Some extremely volatile compounds may have escaped from the samples shortly after collection. Moreover, the analyses may not have detected some compounds in the samples because gas chromatography measures only volatile derivatives. Although only about 20 percent of all substances present in materials of biological origin are volatile enough to be detected with gas chromatography, the compounds which are detected are likely to be involved in mammalian chemoreception (ref 27).

The analytic techniques are limited further if two constituents have the same retention time in the chromatograph, or the same charge-to-mass ratio in the spectrometer. Such compounds are detected as a single substance so the presence of one is masked. However, the probability that two constituents would have the same retention time and charge-to-mass ratio is low.

The relative amounts of compounds separated on the same chromatographic column type can be compared fractionally and across samples. However, fractions separated on different column types cannot be compared because of different chemical response characteristics of the columns. Further, different column types were used at different times during the analysis and sample composition could have changed but was not tested.

## TASTE AND TOXICITY

### BACKGROUND

The qualitative taste information in this section is from experiments and human observations. Some substances, such as indole and skatole, were toxic to terrestrial mammals in laboratory tests (ref 72-91). However, the toxicity is dose-dependent and may be species specific. Kuznetsov used indole and skatole in dolphin chemoreception experiments (ref 6-8).

### RESULTS

Tables 12 through 15 summarize taste and toxicity information on 27 of the principal compounds identified in the marine mammal products tested (ref 92-132). An indole derivative was identified in the basic fractions, so the toxicity of some nitrogenous heterocycles is presented. Taste and toxicity information is presented for acidic, neutral and basic compounds. Compounds for which no relevant information was available are not included in the tables.

### TASTE AND TOXICITY OF SOUR AND/OR BITTER SUBSTANCES

The five compounds listed in table 12 are discussed below.

#### Lactic Acid (ref 92-95, 97)

Lactic acid may be well-suited for chemoreception studies. It is common naturally and occurs in foods such as sauerkraut, pickles, cheese, beer and sour milk. It is considered Generally Recognized As Safe (GRAS) by the US Food and Drug Administration (FDA).

Lactic acid in concentrations from 0.1 to 0.7 percent is a food additive and preservative. Food-grade lactic acid has a mild, fruity flavor resembling yeast, lemon and sour apple. Lactic acid (0.0064 M) was used in taste reception experiments on human subjects.

COMPOUND	FORMULA	MW	AQUEOUS SOLUBILITY	TASTE	TOXICITY	COMMENTS
Lactic Acid	$\text{CH}_3\text{CHOHCO}_2\text{H}$	90.1	Very soluble (92)	Acrid, fruity (93) Salty (94)	Moderate (95)	General purpose food additive GRAS (93,95)
Succinic Acid	$\text{COOH}(\text{CH}_2)_2\text{CO}_2\text{H}$	118.1	Moderate (96)	Tart, bitter (96, 97)	Moderate (95,96, 98)	General purpose food additive (93,95)
Phosphoric Acid	$\text{H}_3\text{PO}_4$	98.0	Very soluble (99)	Sour (100)	Moderate (95)	General purpose food additive (95,100)
Urea	$\text{CO}(\text{NH}_2)_2$	60.1	Very soluble (99)	Bitter, sour (94,101, 102)	Toxic in concentrated solutions (107)	Veterinary nutritional factor diuretic and antiseptic (96,97)
$\beta$ -D-Mannose*	$\text{C}_6\text{H}_{12}\text{O}_6$	180.2	Very soluble (99)	Bitter (99,103,104)	N/A	The $\alpha$ -anomer is sweet (103)

Table 12. Summary of physical properties and toxicity information for sour and/or bitter compounds identified in the urine and semen samples from a male Atlantic bottlenose dolphin, *Tursiops truncatus*, (Tt-583). Parenthetical numbers are literary references. \*While the presence of particular isomer(s) of mannose could not be determined in the analysis, different anomeres of mannose have different tastes.

COMPOUND	FORMULA	MW	AQUEOUS SOLUBILITY	TASTE	TOXICITY	COMMENTS
Glycine	$H_2NCH_2CO_2H$	75.1	Very soluble (95,99)	Sweet (108,109,110)	N/A	Principal amino acid in sugar cane (95) 0.8 times as sweet as sucrose (111)
Glycerol	$HOCH_2CH(OH)CH_2OH$	92.1	Very soluble (109)	Sweet (109)	Low (112)	Food sweetener, about 0.6 times as sweet as cane sugar (96,109)
Inositol	$C_6H_6(OH)_6$	180.2	Very soluble (99)	Sweet (96,105)	N/A	B vitamin (113,114)
Arabitol	$C_5H_7(OH)_5$	152.2	Very soluble (96)	Sweet (96)	N/A	N/A
Erythritol	$C_4H_6(OH)_4$	122.1	Very soluble (96)	Sweet (96)	N/A	About twice as sweet as sucrose (96)
Mannitol	$C_6H_8(OH)_6$	182.2	Very soluble (96)	Sweet (96)	N/A	Excipient and diuretic (96)
Sorbitol	$C_6H_8(OH)_6$	182.2	Very soluble (96)	Sweet (96,115)	Low (96,116)	Food additive, about 0.6 times as sweet as sucrose (95,96)
Xylitol	$C_5H_7(OH)_5$	152.2	Very soluble (99)	Sweet (105,115)	N/A	Has slight unpleasant taste component (115)

Table 13. Summary of physical properties and toxicity information for one sweet amino acid and sweet polyhydric alcohols identified in excretions and secretions of marine mammals. Parenthetical numbers are literary references.

COMPOUND	FORMULA	MW	AQUEOUS SOLUBILITY	TASTE	TOXICITY	COMMENTS
L-Erythrose	$C_4H_8O_4$	120.1	Soluble (96,99)	Sweet (96)	N/A	Syrup (96)
$\alpha$ -D-Galactose	$C_6H_{12}O_6$	180.2	Very soluble in hot water (96,99)	Sweet (105,117,118,119)	N/A	Medical use: liver function test (96,120)
D-Glucose	$C_6H_{12}O_6$	180.2	Very soluble (99)	Sweet (117,118,119,121)	N/A	L-glucose is salty (121); bitter side taste (120); 0.74 times as sweet as sucrose (96)
$\beta$ -Lactose	$C_{12}H_{22}O_{11}$	342.3	Very soluble (99)	Faintly sweet (96,119,121,122)	N/A	Milk sugar; $\beta$ -form sweeter than $\alpha$ -form (96)
$\alpha$ -D-Mannose	$C_6H_{12}O_6$	180.2	Very soluble (96,99)	Sweet (103)	N/A	The $\beta$ -anomer is bitter (103)
D-Xylose	$C_5H_{10}O_5$	150.1	Very soluble (96,99)	Very sweet (96,105,118,123)	N/A	Wood sugar (95,98) diabetic food (96,99)

Table 14. Summary of physical properties and toxicity information for sweet sugars identified in acidic and neutral fractions of excretions and secretions of marine mammals. Parenthetical numbers are literary references. The isomers with the sweetest tastes are listed here, although particular isomers were not specifically identified in the analysis.

COMPOUND	FORMULA	MW	AQUEOUS SOLUBILITY	TASTE	TOXICITY	COMMENTS
p-Hydroxy-3-phenyllactic acid	$C_9H_{10}O_4$	172.2	N/A	N/A	N/A	Experimental carcinogen (95,124)
3,4-Dihydroxybenzoic acid	$C_6H_3(OH)_2CO_2H$	154.1	Slightly soluble (98)	Probably bitter or sour (125)	N/A	Mutagen (116,126,127) Flavor threshold = 30 ppm (106)
3,5-Dihydroxybenzoic acid	$C_6H_3(OH)_2CO_2H$	154.1	Soluble in hot water (99)	Bitter, sour (125,106)	N/A	Flavor threshold = 90 ppm (106) Potential carcinogen (126)
Palmitic Acid	$CH_3(CH_2)_{14}CO_2H$	256.4	Insoluble (98)	N/A	N/A	Tumorigen and mild skin irritant (116)
Stearic Acid	$CH_3(CH_2)_{16}CO_2H$	284.5	Insoluble (99)	N/A	Oral-low IV-high (95,128)	Food additive to 4000 ppm (128)
Oleic Acid	$CH_3(CH_2)_7CH=CH-(CH_2)_7CO_2H$	282.5	Insoluble (99)	Smooth, unpleasant (111)	N/A	
Myristic Acid	$CH_3(CH_2)_{12}CO_2H$	228.4	Insoluble (99)	N/A	Orally non-toxic (129)	Found in nutmeg, palm seeds and milk fats (96)
Arachidic acid	$CH_3(CH_2)_{18}CO_2H$	312.5	Insoluble (99)	N/A	N/A	Found in peanut, vegetable and fish oils (96)
Cholesterol	$C_{27}H_{46}O$	386.7	Insoluble (99)	N/A	N/A	

Table 15. Summary of physical properties and toxicity information for compounds insoluble in water and/or considered unsafe. Parenthetical numbers are literary references.



#### **Succinic Acid (ref 95-98, 115, 124, 130-132)**

Succinic acid has a tart taste, but no odor. Aqueous solutions are slightly bitter and the taste buildup is slow. Succinic acid occurs naturally in broccoli, rhubarb, beets, asparagus, fresh meat extracts and cheese. It is also a general-purpose food additive.

Low concentrations of succinic acid have no systemic toxic effects. It is a common metabolite excreted in human urine.

#### **Phosphoric Acid (ref 96, 97, 100, 133, 134)**

Phosphoric acid, which has a sour taste, occurs naturally in fruits and fruit juices. Phosphoric acid is GRAS; soft drinks contain about 0.01 to 0.05 percent phosphoric acid. Some food processors use phosphoric acid to clarify sugar-bearing juices. In chemoreception experiments, 0.01 to 0.05 percent solutions of phosphoric acid can be used. Inhalation of phosphoric acid vapor should be avoided.

#### **Urea (ref 94, 101, 107)**

Urea has been described as bitter and sour. It is used in veterinary medicine as a nutritional source, as a diuretic and as an antiseptic. Ammonia, a urea derivative, is fatally toxic at a concentration of 0.8 g/liter, which implies that solutions of 2.35-M urea may be harmful if ingested in large quantities. Solutions of 0.82- to 1.0-M urea have been used in human gustation experiments. Weaker solutions (eg, 0.05 M) should be safe for marine mammal studies.

#### **Mannose (ref 96, 99, 102-104, 121)**

Different isomers of mannose have different tastes.  $\beta$ -D-mannose is bitter,  $\alpha$ -D-mannose is sweet and L-mannose is slightly sweet to salty. The specific mannose isomers in the samples were not identified in the analysis. Aqueous mannose solutions have components which produce a complex bitter-sweet stimulus. A solution of ten percent mannose would be appropriate for chemoreception work with marine mammals.

### **TASTE AND TOXICITY OF GLYCINE AND ALCOHOLS**

#### **Glycine (ref 95, 102, 108-111)**

Glycine is the principal amino acid in sugar cane and is common in foods such as gelatin. Most amino acids have a sweet taste. The toxicity of glycine is unknown, but glycine is probably safe as an experimental compound.

**Glycerol** (ref 102, 109, 112, 118, 135)

Glycerol is used commercially as a food sweetener. Data from tests on rats and guinea pigs indicate that the single-dose oral toxicity of glycerol appears to be about half that of ethyl alcohol.

**Inositol** (ref 96, 105, 113, 114)

Inositol (=1, 2, 3, 4, 5, 6-cyclohexanehexol), which has a sweet taste, is a B-complex vitamin found in wheat germ, lecithin, whole grains, milk, molasses, citrus fruit and brewers yeast. For medicinal uses, daily oral doses of 0.5 to 1.0 grams can be maintained for humans. Test solutions below 0.02 M should be safe.

**Arabitol** (ref 96)

Arabitol (=1, 2, 3, 4, 5-pentanepentol) has a sweet taste, but toxicity information was unavailable.

**Erythritol** (ref 96)

Erythritol (=1, 2, 3, 4-butanetetrol) is about twice as sweet as sucrose; no oral toxicity is known. Intravenously-administered erythritol was toxic in dogs in the amount of 5 g per kilogram of body weight.

**Mannitol** (ref 96, 135)

Mannitol (=mannite or manna sugar), which has a sweetish taste, is used as a pharmaceutical excipient and as a diuretic and diagnostic aid for the kidney function. Oral toxicity data were not available in the literature; however, mannitol should be safe for marine mammal chemoreception studies.

**Sorbitol** (ref 95, 96, 115, 116, 118, 135)

Sorbitol (=D-glucitol), which is about 60 percent as sweet as sucrose, is found in many berries such as ripe mountain ash, as well as in cherries, plums, pears, apples, seaweed, algae and in blackstrap molasses. Sorbitol is used as a sweetening agent and tablet excipient in drugs. It also has various uses in veterinary medicine. Solutions of up to 2.8-M sorbitol were used in human taste perception experiments, and similar concentrations should be appropriate for marine mammals.

## Xylitol (ref 115)

Xylitol (=1, 2, 3, 4, 5-pentanepentol), has a sweet, syrupy taste and possible sour aftertaste. Taste experiments on human subjects used 1.0-M xylitol. Similar concentrations should not harm marine mammals.

## TASTE OF SWEET SUGARS

### General Comments (ref 119, 121, 136)

The particular isomers of the sugars listed in table 14 were not determined in the analysis, but the isomers with the sweetest tastes are listed. The sugars in table 14 are also polyhydric alcohols, but differ in chemical structure and properties from those in table 13. The slow aqueous interconversion between the  $\alpha$ - and  $\beta$ -forms of sugar suggests that sugar solutions should equilibrate for at least three hours before use in chemoreception experiments. Equilibrated solutions will produce uniform stimulus quality. The sugars listed in table 14 are not considered harmful in normal use.

### Erythrose (ref 96)

D-erythrose (=R)-2, 3, 4-trihydroxybutanal) is described as a syrup, and L-erythrose, as a sweet tasting syrup. Toxicity information was unavailable for erythrose.

### Galactose (ref 96, 117-120)

Galactose, which has a sweet taste, is found in many foods. A dosage of 40 g of galactose has been used as a liver function test.

In taste tests on humans, 1.5-M solutions of galactose (roughly 27 percent) were used. This concentration also should be safe for gustation experiments on marine mammals.

### Glucose (ref 96, 115, 117-119, 121, 136, 137)

Glucose is an important nutrient commonly found in foods and generally is described as sweet. Glucose was observed by some researchers to produce a bitter side taste. L-glucose is slightly salty. A solution of 2.0-M-glucose (roughly 36 percent) was used in taste tests on human subjects and should be safe for marine mammal chemoreception experiments.

### Lactose (Ref 96, 117-119, 122)

Lactose is faintly sweet but not as sweet as the other

sugars in table 14. Solutions of 0.6-M lactose (about 21 percent) were used as stimuli for human chemoreception studies and the same concentration is suggested for experiments on marine mammals.

### **Mannose**

Mannose was discussed above in the section on Taste and Toxicity of Sour and/or Bitter Substances.

### **Xylose (ref 96, 115, 118, 123)**

D-Xylose has a very sweet taste tinged by slightly unpleasant components in its taste quality. It is used as a diagnostic aid and as a diabetic food. Xylose concentrations up to 2.5 M have been used in human perceptual studies; similar concentrations should be safe for marine mammals.

### **TOXICITY AND DISCUSSION OF COMPOUNDS WHICH ARE INSOLUBLE IN WATER AND/OR UNSAFE FOR CHEMORECEPTION EXPERIMENTS**

The compounds listed in table 15 are inappropriate for marine mammal chemoreception experiments. p-Hydroxy-3-phenyllactic, 3,4-dihydroxybenzoic and palmitic acids are carcinogenic, mutagenic and tumorigenic, respectively (ref 95, 99, 116, 124, 126, 127). Because tests suggested that aromatic diols generally should be considered potential mutagens and carcinogens (ref 126), 3,5-dihydroxybenzoic acid also may be unsafe. Stearic, oleic, myristic and arachidic acids and cholesterol are insoluble in water (ref 96, 99).

### **TOXICITY OF NITROGEN HETEROCYCLES**

The basic fractions contained several nitrogenous compounds, including tryptamine, an indole derivative. Indole and skatole (3-methylindole), which have strong odors, are common metabolic products (ref 138-144). They become toxic to mammals at species-dependent dosages (ref 72-91).

Sokolov et al used a 0.01-percent solution of indole in seawater for chemoreception experiments with dolphins, but does not mention toxic reactions (ref 7). Cattle have been given 0.05 grams of indole per kilogram of body weight without ill effects (ref 74). Indole should be handled with great care. Indole concentrations for marine mammal experiments should not exceed 0.1 percent (ref 91).

Skatole was more toxic than indole when administered orally to cattle (ref 74), but an exact mammalian toxicity threshold was unreported.

## RECOMMENDED EXPOSURE

Table 16 summarizes toxicity and exposure information on compounds identical or similar to those found in the samples. The listed safe levels are maxima. The levels are probably conservative, however, because considerable ingestion of the solution is assumed whereas ingestion volumes of test solutions have not been measured so far in marine mammal chemoreception studies. Also, the actual toxicities of these chemicals to marine mammals are unknown.

Apart from Soviet reports, little information is available on systematic exposure of marine mammals to chemicals.

PREVIOUS PAGE  
IS BLANK



COMPOUND	GENERAL pH RANGE	ESTIMATED MAXIMUM SAFE LEVEL OF ORAL EXPOSURE	LITERARY REFERENCE
Lactic Acid	Acidic	0.7%	93
Succinic Acid	Acidic	Not found	
Phosphoric Acid	Acidic	0.05%	100
Urea	Basic	0.05M	94, 101 107
Mannose	Neutral	10% (Non-toxic)	103
Glycine	Acidic	2.0M (Non-toxic)	109
Glycerol	Acidic	Non-toxic	112
Inositol	Acidic-Neutral	0.02M	113
Arabitol	Neutral	Not found	
Erythritol	Neutral	Not found	
Mannitol	Neutral	Non-toxic	
Sorbitol	Neutral	2.8M	118
Xylitol	Neutral	1.0M (Non-toxic)	115
Erythrose	Neutral	Non-toxic	
Galactose	Neutral	2.5M (Non-toxic)	118
Glucose	Neutral	2.0M (Non-toxic)	118
Lactose	Neutral	0.6M (Non-toxic)	118
Xylose	Neutral	2.5M (Non-toxic)	118
Indole	Basic	0.01%	74
Skatole	Neutral-Basic	0.005%	74

Table 16. Recommended concentrations of compounds for use in chemoreception experiments.

For future work with the compounds discussed here, the following points are relevant to the NOSC chemoreception program:

1. The values listed in table 16 are estimated maximum concentrations for oral exposure to marine mammals.
2. Marine mammals' exposure to chemicals toxic to other animals should be limited. If greater concentrations are used, the physical condition of the test animals should be monitored closely.
3. Test mannose, erythrose, glycerol, erythritol, arabinol and mannitol for taste reception using solutions of 10 percent.
4. The chemicals listed in tables 12 through 14 are considered suitable for use in chemoreception experiments.
5. Phosphoric acid is probably also suitable for chemoreception experiments, but requires special handling and storage (ref 145). Phosphoric acid should not be used, therefore, if other chemicals are available (ref 145).
6. p-Hydroxy-3-phenyllactic, 3,4-dihydroxybenzoic, and palmitic acids are hazardous to humans and should not be used in marine mammal chemoreception studies.
7. Because related aromatic diols are mutagens (ref 126, 107), use of 3,5-Dihydroxybenzoic acid in marine mammal chemoreception studies is not recommended.
8. Stearic, oleic, myristic and arachidic acids and cholesterol are unsuitable for marine mammal chemoreception studies because they are insoluble in water.
9. Monitor animal health carefully throughout experiments. Avoid prolonged exposure to known toxic compounds, even in very dilute solutions.

## REFERENCES

1. Gorman, ML, Nedwell, DB and Smith, RM, An Analysis of the Contents of the Anal Scent Pockets of Herpestes auropunctatus (Carnivora: Viverridae), J Zool, London, vol 172, p 389-399, 1974
2. Gorman, ML, Jenkins, D and Harper, RS, The Anal Scent Sacs of the Otter (Lutra lutra, J Zool, London, vol 186, p 463-474, 1978
3. NOSC TR 353, Chemoreception in Marine Mammals: A Review of the Literature, by WR Lowell and WF Flanigan, Jr, p 4-9, Nov 1978
4. Lowell, WR and Flanigan, WF, Jr, Marine Mammal Chemoreception, Mammal Review, vol 10(1), p 53-59, 1980
5. Winn, HE and Schneider, J, Communication in Sireniens, Sea Otters and Pinnipeds (Communication in Selected Groups), In: How Animals Communicate, TA Sebeok, ed, chap 32, p 809-840, Indiana University Press, 1978
6. Ridgway, SH, Mammals of the Sea: Biology and Medicine, p 479-484, Charles C Thomas, 1972
7. Sokolov, VE and Kuznetsov, VB, Chemoreception in the Black Sea Dolphin Tursiops truncatus Mont, Doklady Akademn Nauk SSSR Ser Biol, vol 201(1-6), p 768-770, Moscow, 1971-72
8. Kuznetsov, VB, A Method of Studying Chemoreception in the Black Sea Bottlenose Dolphin (Tursiops truncatus), In: Morfologiya, Fisiologiya i Akustika Morskikh Mlekopitayushchikh, Sokolov, VY, ed, p 27-45, Izdatel'stvo "Nauka", Moscow, 1974
9. Bullock, TH and Gurevich, VS, Soviet Literature on the Nervous System and Psychobiology of Cetacea, Internat Rev Neurobio, vol 21, p 47-59, 1979
10. Kuznetov, VB, Chemoreception in Dolphins of the Black Sea: Aphalines (Tursiops truncatus Mont) Common Dolphins (Delphinus delphis L) and Porpoises (Phocoena phocoena L), Doklady Akademii Nauk SSSR, vol 249(6), p 1498-1500, 1979





11. Stock, R and Rice, CBF, Chromatographic Methods, 2nd ed, Chapman and Hall, Ltd, London, Eng, 1967
12. Allinger, NL, Cava, MP, DeJongh, DC, Johnson, CR, Level, NA and Stevens, CL, Organic Chemistry, p 181, Worth Publishers, Inc, New York, 1971
13. Fennessey, PV and Tjoa, SS, Pilot-Study Analysis of Physiological Material from the Sea Lion and Dolphin, NOSC CR in preparation, 1983
14. Alm, J, Havenfeldt, L and Larsson, A, Concentrations of Organic Acids in the Urine of Healthy Newborn Children, Ann of Clin Biochem, vol 15(5), p 245-249, 1978
15. Bjorkman, L, McLean, C and Steen, G, Organic Acids in Urine from Human Newborns, Clin Chem, vol 22(1), p 49-52, 1976
16. Lawson, AM, Chalmers, RA and Watts, RWE, Urinary Organic Acids in Man. I. Normal Patterns, Clin Chem, vol 22(8), p 1283-1287, 1976
17. Chalmers, RA, Healy, MJR, Lawson, AM and Watts, RWE, Urinary Organic Acids in Man. II. Effects of Individual Variation and Diet on the Urinary Excretion of Acidic Metabolites, Clin Chem, vol 22(8), p 1288-1291, 1976
18. Chalmers, RA, Healy, MJR, Lawson, AM, Hart, JT and Watts, RWE, Urinary Organic Acids in Man. III. Quantitative Ranges and Patterns of Excretion in a Normal Population, Clin Chem, vol 22(8), p 1292-1298, 1976
19. Knights, BA, Legendre, M, Laseter, JL and Storer, JS, Use of High-Resolution Open Tubular Class Capillary Columns to Separate Acidic Metabolites in Urine, Clin Chem, vol 21(7), p 888-889, 1975
20. Watts, RWE, Chalmers, RA and Lawson, AM, Abnormal Organic Acidurias in Mentally Retarded Patients, The Lancet, p 368-371, 15 Feb 1975
21. Thompson, JA, Markey, SP and Fennessey, PV, Gas-Chromatographic/Mass-Spectrometric Identification and Quantitation of Tetrionic and Deoxytetrionic Acids in Urine from Normal Adults and Neonates, Clin Chem, vol 21(13), p 1892-1898, 1975

22. Bindel, TH, Fennessey, PV, Miles, BS and Goodman, SI, 4-Hydroxycyclohexane-1-Carboxylic Acid: An Unusual Compound Isolated from the Urine of Children with Suspected Disorders of Metabolism, *Clinica Chimica Acta*, vol 66, p 209-217, 1976
23. Linstedt, S, Norberg, K, Steen, G and Wahl, E, Structure of Some Aliphatic Dicarboxylic Acids Found in the Urine of an Infant with Congenital Lactic Acidosis, *Clin Chem*, vol 22(8), p 1330-1338, 1976
24. Petterson, JE and Stokke, O, Branched Short-Chain Dicarboxylic Acids in Human Urine, *Biochimica et Biophysica Acta*, vol 304, p 316-325, 1973
25. Mamer, OA and Tjoa, SS, 2-Ethylhydracrylic Acid: A Newly Described Urinary Organic Acid, *Clinica Chimica Acta*, vol 55, p 199-204, 1974
26. Jellum, E, Storseth, P, Alexander, J, Helland, P, Stokke, O and Teig, E, Application of Glass Capillary-Column Gas Chromatography-Mass Spectrometry to the Studies of Human Diseases, *J of Chromat*, vol 126, p 487-493, 1976
27. Jellum, E, Profiling of Human Body Fluids in Healthy and Diseased States Using Gas Chromatography and Mass Spectrometry, with Special Reference to Organic Acids, *J of Chromat*, vol 143, p 427-462, 1977
28. Young, DS, Epley, JA and Goldman, P, Influence of a Chemically Defined Diet on the Composition of Serum and Urine, *Clin Chem*, vol 17(8), p 765-773, 1971
29. Armstrong, MD and Shaw, KNF, The Occurrence of (-)- $\beta$ -m-Hydroxyphenylhydracrylic Acid in Human Urine, *J Biol Chem*, vol 225, p 269-278, 1957
30. Duran, M, Gompertz, D, Bruinvis, L, Ketting, D and Wadman, SK, The Variability of Metabolite Excretion in Propionicacidaemia, *Clinica Chimica Acta*, vol 82, p 93-99, 1978
31. Mee, JML, Rapid Determination of  $\beta$ -Hydroxybutyric Acid in Blood and Milk by Gas Chromatography, *J of Chromatog*, vol 101, p 414-416, 1974
32. Kuhara, T and Matsumoto, I, Studies on the Urinary Acidic Metabolites from Three Patients with Methylmalonic Aciduria, *Biomed Mass Spectrom*, vol 7(10), p 424-428, 1980

33. Goodman, SI, McCabe, ERB, Fennessey, PV, Miles, BS, Mace, JW and Jellum, E, Methylmalonic/  $\beta$ -Hydroxy-n-Valeric Aciduria Due to Methylmalonyl-CoA Mutase Deficiency, *Clinica Chimica Acta*, vol 57, p 441-449, 1978
34. Goodman, SI, Organic Aciduria in the Riboflavin-Deficient Rat, *American J Clinical Nutrition*, vol 34(11), p 2434-2437, 1981
35. Jellum, E, Stokke, O and Eldjarn, L, Application of Gas Chromatography, Mass Spectrometry, and Computer Methods in Clinical Biochemistry, *Analyt Chem*, vol 45(7), p 1099-1106, June 1973
36. Lewis, S, Kenyon, CN, Meili, J and Burlingame, AL, High Level Gas Chromatographic/Real-Time High Resolution Mass Spectrometric Identification of Organic Acids in Human Urine, *Analyt Chem*, vol 51(8), p 1275-1285, July 1979
37. Gates, SC, Dendramis, N and Sweeley, CC, Automated Metabolic Profiling of Organic Acids in Human Urine, *Clin Chem*, vol 24(10), p 1674-1679, 1978
38. Thompson, JA, Miles, BS and Fennessey, PV, Urinary Organic Acids by Age Groups in a Healthy Pediatric Population, *Clin Chem*, vol 23(9), p 1734-1738, 1977
39. Tanaka, K, Hine, DG, West-Dull, A and Lynn, TB, Gas-Chromatographic Method of Analysis for Urinary Organic Acids. 1. Retention Indices of 155 Metabolically Important Compounds, *Clin Chem*, vol 26(13), p 1839-1846, 1980
40. Fornstedt, N, A Method for High-Performance Liquid Chromatographic Screening of UV-Positive Components in Urine Eluate from Sephadex G-10 and Modifications for Determination of Urinary Salicylic Salicyluric and Gentisic Acids, *J Chromatography*, vol 230(2), p 253-261, 1982
41. Mortensen, PB, Urinary Excretion of  $C_4$ - $C_{10}$ -Dicarboxylic Acids and Antiketogenic Properties of Adipic Acid in Ketogenic-Stimulated Rats Due to Diabetes, Long-Chain and Short-Chain Monocarboxylic Acids, *Biochimica et Biophysica Acta*, vol 664(2), p 335-348, 1981
42. Paz, GF, Sofer, A, Homonnai, ZT and Kraicer, PF, Human Semen Analysis: Seminal Plasma and Prostatic Fluid Compositions and their Interrelations with Sperm Quality, *Int J Fertil*, vol 22(3), p 140-147, 1977

43. Homonnai, ZT, Matzkin, H, Fainman, N, Paz, G and Kraicer, PF The Cation Composition of the Seminal Plasma and Prostatic Fluid and its Correlation to Semen Quality, Fertility and Sterility, vol 29(5), p 539-542, 1978
44. Mann, T, Evaluation of Semen By Chemical Analysis, Chap 3 in: The Artificial Insemination of Farm Animals, EJ Perry, ed, 3rd ed, p 32-44, Rutgers Univ Press, 1960
45. Shulman, S and Bronson, P, Immunochemical Studies on Human Seminal Plasma. I. Changes in Composition During Storage, as Demonstrated by Electrophoresis, Fertility and Sterility, vol 19(4), p 549-561, Jul-Aug 1968
46. Jayaraman, S, Hurkadli, KS and Sheth, AR, Lack of Seasonal Changes in Biochemical Constituents of Bonnet Monkey Semen, Arch of Androl, vol 4, p 327-330, 1980
47. Robertson, IS, Wilson, JC and Morris, PGD, Growth in Castrated Cattle, The Vet Rec, vol 81(4), p 88-103, 22 Jul 1967
48. Banerjee, AK and Ganguli, NC, Some Aspects of The Composition of Buffalo Semen, J Reprod and Fertl, vol 33(1), p 171-173, 1973
49. Mann, T, Biochemistry of Stallion Semen, J Reprod and Fertl, Suppl 23, p 47-52, Oct 1975
50. Holtz, W and Foote, RH, Composition of Rabbit Semen and the Origin of Several Constituents, Biol of Reprod, vol 18(2), p 286-292, 1978
51. Brown-Woodman, PDC and White, IG, Amino Acid Composition of Semen and the Secretions of the Male Reproductive Tract, Aust J Biol Sci, vol 27(4), p 415-422, 1974
52. Paulsen, H, Nissen, HP, Heinze, I, Schirren, C and Kreysel, HW, Zusammensetzung der Proteine des Humanspermas bei Oligozoo-Steratozoo-, Asthenozoo- and Azospermie, Andrologia, vol 12, p 61-65, 1980
53. Nissen, HP, Schirren, C, Kreysel, HW and Heinze, I, Verteilung der Aminosauern im Humansperma unter diagnostischen Gesichtspunkten, Andrologia, vol 11(2), p 109-112, 1979
54. van der Horst, CJG and Bol, JJ, Sterility of Three Brothers in Connection with a Disturbed Carbohydrate Metabolism in Semen, Andrologia, vol 7(1), p 15-24, 1975

55. Chulavatnatol, M and Ruenwongsa, P, Activation of Proenzyme of Acidic Protease from Human Seminal Plasma, *Biochimica et Biophysica Acta*, vol 452, p 525-532, 1976
56. van der Horst, CJG, Elgersma, A, deBoer, MJ and Bestebroer, AC, Some Biochemical Parameters for Qualification of Bull Semen, *The Vet Qtrly*, vol 1(2), p 97-108, April 1979
57. Cechova, D, Jonakova, V, Havranova, M, Sedlakova, E and Mach, O, Isolation of Acidic Acrosin Isoinhibitors (BUSI I A, BUSI IBI and BUSI IB2) from Bull Seminal Plasma, *Hoppe-Seyler's Z Physiol Chem*, vol 360(12), p 1759-1766, December 1979
58. Cechova, D, Jonakova, V, Sedlakova, E and Mach, O, Isolation of Basic Acrosin Inhibitor from Bull Seminal Plasma (BUSI II), *Hoppe-Seyler's Z Physiol Chem*, vol 360(12), p 1753-1758, December 1979
59. Chattaway, FW, Wheeler, PR and O'Reilly, J, Purification and Properties of Peptides which Induce Germination and Blastospores of Candida albicans, *J Gen Microbiol*, vol 120, p 431-437, 1980
60. D'Alossio, G, DiDonato, A, Furia, A, Leone, E, Libonati, M, Parante, A and Suzuki, H, Bull Semen RNAase Revisited, *J Mol Biol*, vol 146(2), p 269-274, 1981
61. Kysilka, C, Haemolytic Factor from Bull Seminal Vesicle Fluid: Chemical and Biochemical Properties, Amino Acid Composition, *Int J Peptide Prot Res*, vol 4(5), p 303-307, 1972
62. Johnson, LA, Pursel, VG, Gerrits, RJ and Thomas, CH, Free Amino Acid Composition of Porcine Seminal, Epididymal and Seminal Vesicle Fluids, *J Anim Sci*, vol 34(3), p 430-434, March 1972
63. Ahluwalia, B and Holman, RT, Fatty Acid Composition of Lipids of Bull, Boar, Rabbit and Human Semen, *J Reprod Fert*, vol 18, p 431-437, 1969
64. Glenn, GC, The CAP Urine Chemistry Survey Program for 1977, *Am J Clin Path*, vol 70(3), p 513-515, 1978
65. Anderson, RA, Jr, Reddy, JM, Oswald, C and Zaneveld, LJD, Enzymic Determination of Fructose in Seminal Plasma by Initial Rate Analysis, *Clin Chem*, vol 25(10), p 1780-1782, October 1979

66. Bushway, AA, Clegg, ED and Keenan, TW, Composition and Synthesis of Gangliosides in Bovine Testis, Sperm and Seminal Plasma, Biol of Reprod, vol 17(3), p 432-442, 1977
67. Bushway, AA, Bouchard, BG, Engdahl, G, Keenan, TW and Bushway, RJ, Composition of Gangliosides in Ovine Testis, Comp Biochem Physiol, vol 68B, p 245-250, 1981
68. Mokkaapati, S and Dominic, CJ, The Accessory Reproductive Glands of Five Male Mammals, Proc Zool Soc Calcutta, vol 30(172), p 1-5, 1977
69. Caldwell, DK and Caldwell, MC, Cetaceans (Communication in Selected Groups) In: How Animals Communicate, TA Sebeok, ed, chap 31, p 805, Indiana University Press, 1978
70. Jones, TR and Plakke, RK, The Histology and Histochemistry of the Perianal Scent Gland of the Reproductively Quiescent Black-Tailed Prairie Dog (*Cynomys Ludovicianus*), J Mamm, vol 62(2), p 362-368, 1981
71. Albone, ES and Fox, MW, Anal Gland Secretion of the Red Fox, Nature, London, vol 233, p 569-570, 1971
72. Roe, DA, Effects of Methionine and Inorganic Sulfate on Indole Toxicity and Indican Excretion in Rats, J Nutrit (US), vol 105(1), p 645-653, May 1971
73. Nakonecza, I, Forbes, JC and Rogers, KS, The Arthritogenic Effect of Indole, Skatole and Other Tryptophan Metabolites in Rabbits, Am J Path, vol 57(3), p 523-538, December 1969
74. Hammond, AC, Carlson, JR and Breeze, RG, Indole Toxicity in Cattle, Vet Rec, vol 107(15), p 344-346, 1980
75. Carlson, JR, Dickinson, EO, Yokoyama, MT and Bradley, B, Pulmonary Edema and Emphysema in Cattle After Intraruminal and Intravenous Administration of 3-Methylindole, Am J Vet Res, vol 36(9), p 1341-1347, September 1975
76. Yokoyama, MT, Carlson, JR and Dickinson, EO, Ruminal and Plasma Concentrations of 3-Methylindole Associated with Tryptophan-Induced Pulmonary Edema and Emphysema in Cattle, Am J Vet Res, vol 36(9), p 1349-1352, September 1975

77. Pirie, HM, Breeze, RG, Selman, IE and Wiseman, A, Indole-Acetic Acid, 3-Methyl Indole and Type 2 Pneumocyte Hyperplasia in a Proliferative Alveolitis of Cattle, Vet Rec, vol 98(13), p 259-260, 27 March 1976
78. Hammond, AC, Bray, TM, Cummins, KA, Carlson, JR and Bradley, BJ, Reduction of Ruminal 3-Methylindole Production and the Prevention of Tryptophan-Induced Acute Bovine Pulmonary Edema and Emphysema, Am J Vet Res, vol 39(9), p 1404-1406, 1978
79. Hammond, AC, Carlson, JR and Breeze, RG, Prevention of Tryptophan-Induced Acute Bovine Pulmonary Oedema and Emphysema (Fog Fever), Vet Rec, vol 107(14), p 322-325, 1980
80. Cornelius, LM, Coulter, D, Doster, A and Rawlings, C, Pathophysiologic Studies of Calves Given 3-Methylindole Intraruminally, Am J Vet Res, vol 40(4), p 571-575, 1979
81. Hanafy, MSM and Bogan, JA, The Covalent Binding of 3-Methylindole Metabolites to Bovine Tissue, Life Sciences, vol 27(13), p 1225-1231, 1980
82. Huang, TW, Carlson, JR, Bray, TM and Bradley, BJ, 3-Methylindole Induced Pulmonary Injury in Goats, Am J Path, vol 87(3), p 647-666, 1977
83. Bray, TM and Carlson, JR, Role of Mixed Function Oxidase in 3-Methylindole-Induced Acute Pulmonary Edema in Goats, Am J Vet Res, vol 40(9), p 1268-1272, September 1979
84. Bradley, BJ and Carlson, JR, Ultrastructural Pulmonary Changes Induced by Intravenously Administered 3-Methylindole in Goats, Am J Path, vol 99(2), p 551-560, June 1980
85. Bradley, BJ, Carlson, JR and Dickinson, EO, 3-Methylindole-Induced Pulmonary Edema and Emphysema in Sheep, Am J Vet Res, vol 39(8), p 1355-1358, 1978
86. Yang, NYJ and Carlson, JR, Release of Marker Lysosomal Enzymes by 3-Methylindole and Indole from Rabbit Lung Lavage Cells (39652), Proc Soc Exper Biol Med, vol 154, p 269-273, 1977
87. Bray, TM and Carlson, JR, Tissue and Subcellular Distribution and Excretion of 3(<sup>14</sup>C) Methylindole in Rabbits after Intratracheal Infusion, Can J Physiol Pharm, vol 58(12), p 1399-1405, 1980

88. Agarwal, AK, Gupta, ML and Bhargava, KP, Pharmacological Evaluation of Some Newer Substituted Indoles as Antiovolatory Agents, Ind J Med Res, vol 66(6), p 983-986, December 1977
89. Snell, K, Hypoglycaemia Caused by Indole and Quinoline Derivatives, Trans Biochem Soc London, vol 7(4), p 745-749, 1979
90. Bosin, TR, Campaigne, E, Dinner, A, Rogers, RB and Maickel, RP, Comparative Toxicological Studies of Indole, Benzo (b) Thiophene, and 1-Methylindole Derivatives, J Tox Environ Health, vol 1(3), p 515-520, 1976
91. Sgibnev, AK and Orlova, TA, Problem of Studying the Toxicity of Indole, Problemy Kasmicheskai Biologii, vol 16, p 130-135, 1971
92. USDL OSHA Material Data Sheet, Lactic Acid, 50%, Form OSHA 20, Monsanto, St Louis
93. Monsanto Technical Brochure O/FI-2A, Water White Food Grade Lactic Acid, Monsanto, St Louis, 1971
94. McBurney, DH and Shick, TR, Taste and Water Taste of Twenty-six Compounds for Man, Perception and Psychophysics, vol 10(4A), p 249-252, 1971
95. Sax, MI, Dangerous Properties of Industrial Materials, Fifth edition, Van Nostrand Reinhold Company, New York, 1979
96. The Merck Index: An Encyclopedia of Chemicals and Drugs, 9th ed, Merck and Co Inc, Rahway, NJ, 1976
97. Furia, TE, ed, Handbook of Food Additives, 2nd ed, CRC Press, The Chemical Rubber Co, Cleveland, OH, 1972
98. Patty, F, Industrial Hygiene and Toxicology, 2nd ed, vol 2, Wiley & Sons, 1963
99. Weast, RC, CRC Handbook of Chemistry and Physics, 50th ed, The Chemical Rubber Company, 1970
100. Monsanto Technical Bulletin IC/DP-239, Industry Guide to the Profitable Use of Monsanto Phosphoric Acid, Monsanto, St Louis, 1976
101. McBurney, DH, Smith, DV and Shick, TR, Gustatory Cross Adaptation: Sourness and Bitterness, Perception and Psychophysics, vol 11(3), p 228-232, 1972



102. Parker, GH, Smell, Taste and Allied Senses in the Vertebrates, (Monographs on Experimental Biology), Philadelphia and London, JB Lippincott Co, 1922
103. Steinhardt, RG, Calvin, AD and Dodd, EA, Taste-Structure Correlation with  $\alpha$ -D-Mannose and  $\beta$ -D-Mannose, Science, vol 135, p 367-368, 1962
104. Boyd, WC and Matsubara, S, Different Tastes of Enantiomorphic Hexoses, Science, vol 137, p 669, 1962
105. Ney, KH, Bitterness of Peptides: Amino Acid Composition and Chain Length, from Food Taste Chemistry, ACS Symposium Series 115, Boudreau, JC, ed, p 167, American Chemical Society, Washington, DC, 1979
106. Maga, JA and Lorepz, K, Taste Threshold Values for Phenolic Acids Which Can Influence Flavor Properties of Certain Flours, Grains and Oilseeds, Cereal Science Today, Cereal Foods World, vol 18(10), p 326-328, 350, 1973
107. Maynard, LA, Loosli, JK, Hintz, HF and Warner, RG, Animal Nutrition Seventh Edition, p 51, 165-166, McGraw-Hill Book Co, 1979
108. Pfaffmann, C, The Sense of Taste, Chapter XX in: Handbook of Physiology: Neurophysiology, vol I, J Field, HW Macoun and VE Hall, ed, Williams and Wilkins, Pub, Baltimore, 1959
109. Cayne, BS, Glycerol, Glycine, Encyclopedia Americana, vol 12, p 819, Americana Corp, New York, 1976
110. Meiselman, HL and Halpern, BP, Effects of Gymnema Sylvestre on Complex Tastes Elicited by Amino Acids and Sucrose, Physiology and Behavior, vol 5, p 1379-1384, 1970
111. Shiffman, SS and Dackis, C, Taste of Nutrients: Amino Acids, Vitamins and Fatty Acids, Perception and Psychophysics, vol 17(2), p 140-146, 1975
112. Smyth, HF, Jr, Seaton, J and Fischer, L, The Single Dose Toxicity of Some Glycols and Derivatives, J Indust Hyg Toxicol, vol 23(6), p 259-268, June 1941
113. Airola, P, How to Get Well, p 266, Health Plus, 1974
114. Davis, A, Let's Eat Right to Keep Fit, p 71-72, New American Library, 1970

115. Shiffman, SS, Reilly, DA and Clark, TB, Qualitative Differences Among Sweeteners, Physiology and Behavior, vol 23, p 1-9, 1979
116. National Institute for Occupational Safety and Health, Registry of Toxic Effects of Chemical Substances, 1975 ed, Public Health Service Center for Disease Control, US Department of Health, Education and Welfare, US Government Printing Office, Washington, DC, 1975
117. Daimant, H, Oakley, B, Strom, L, Wells, C and Zotterman, Y, A Comparison of Neural and Psychophysical Responses to Taste Stimuli in Man, Acta Physiol Scand, vol 64, p 67-74, 1965
118. Moskowitz, HR, Perceptual Attributes of the Taste Sugars, J Food Science, vol 37, p 624-626, 1972
119. Pangborn, RM and Gee, SC, Relative Sweetness of  $\alpha$ - and  $\beta$ -Forms of Selected Sugars, Nature, vol 191(4790), p 810-811, 1961
120. The Merck Index: An Encyclopedia of Chemicals and Drugs, 7th ed, Merck and Co Inc, Rahway, NJ, 1960
121. Beidler, LM, Chemical Extraction of Taste and Odor Receptors, from Flavor Chemistry, Advances in Chemistry Series 56, RF Gould, ed, p 11, American Chemical Society, Washington DC, 1966
122. Fabian, FW and Blum, HB, Relative Taste Potency of Some Basic Food Constituents and Their Competitive and Compensatory Action, Food Research, vol 8(3), p 179-193, 1943
123. Bartoshuk, LM, Gentile, RT, Moskowitz, HR and Meiselman, HL, Sweet Taste Induced by Miracle Fruit (Synsepalum dulcificum), Physiology and Behavior, vol 12, p 449-456, 1974
124. Zharova, EI, Sergeeva, TI, Makhalova, NV, Romanenko VI; Chitiridi, NG and Raushendakh, MO, Transplacental Carcinogenic Action of p-Hydroxyphenyl-Lactic Acid, Lab Syst Bod Dis, Oncol Sci Ctr, Acad Med Sci USSR, Moscow, Trans fm: Byul Eksper Biol i Med, vol 87(1), p 39-41, January 1979, Plenum, 1979
125. Belitz, HD, Chen, W, Jugel, H, Treleano, R, Wieser, H, Gasteiger, J and Marsili, M, Sweet and Bitter Compounds: Structure and Taste Relationship, from Food Taste Chemistry, ACS Symposium Series 115, Boudreau, JC, ed, American Chemical Society, p 114, Washington, DC, 1979

126. Yang, CY and Klemencic, JM, Mutagenity and Carcinogenicity of Polyhydric Phenols, American Association of Cancer Research, Proceedings, vol 20, 473, p 117, 1979
127. Stich, HF, Rosin, MP, Wu, CH and Powrie, WD, The Action of Transition Metals on the Genotoxicity of Simple Phenols, Phenolic Acids and Cinnamic Acids, Cancer Letters, vol 14(3), p 251-260, 1981
128. Opdyke, DLJ, Monographs on Fragrance Raw Materials, Stearic Acid, Fd Cosmet Toxicol, vol 17, p 383-388, 1979
129. Gleason, MN, Gosselin, RE and Hodge, HC, Clinical Toxicology of Commercial Products, Acute Poisoning (Home & Farm), p 83, Williams & Wilkins Co, Baltimore, 1963
130. Gosselin, RE, Hodge, HC, Smith, RP and Bleason, MN, Clinical Toxicology of Commercial Products, 4th ed, Williams and Wilkins, Baltimore, MD, 1976
131. Sax, MI, Dangerous Properties of Industrial Materials, 4th ed, Van Nostrand Reinhold, 1975
132. International Labour Office, Encyclopedia of Occupational Health and Safety, vol 2, McGraw-Hill Book Co, New York, 1971
133. Toxic and Hazardous Chemicals in Industry, Wall Poster Chart, Science Related Materials, Inc, Pub, Jamesville, WI, 1980
134. American Conference of Governmental Industrial Hygienists, Documentation of the Threshold Limit Values for Substances in Workroom Air, 3rd ed, Am Conf Gov Ind Hyg, Cincinnati, OH, 1971 (plus suppl)
135. Beck, FF, Carr, CJ and Krantz, JC, Jr, Acute Toxicity of Certain Sugar Alcohols and Their Anhydrides, Soc Expt'l Biol & MedProc, vol 35, p 98-99, 1936
136. Schiffman, SS and Erickson, RP, A Theoretical Review A Psychophysical Model for Gustatory Quality, Physiology and Behavior, vol 7, p 617-633, 1971
137. Moskowitz, HR, Perceptual Changes in Taste Mixtures, Perception & Psychophysics, vol 11(4), p 257-262, 1972
138. Schaefer, J, Sampling, Characterization and Analysis of Malodours, Agriculture and Environment, vol 3, p 121-127, 1977

139. Lunn, F and VanDeVyver, J, Sampling and Analysis of Air in Pig Houses, Agric and Environ, vol 3, p 159-169, 1977
140. Yasuhara, A, Relation Between Odor and Odorous Components in Solid Swine Manure, Chemosphere, vol 9, p 587-592, 1980
141. Yasuhara, A and Fuwa, K, Odor and Volatile Compounds in Liquid Swine Manure. II. Steam-distillable Substances, Bull Chem Soc Japan, vol 50(11), p 3029-3032, 1977
142. Yasuhara, A and Fuwa, K, Odor and Volatile Compounds in Liquid Swine Manure. III. Volatile and Odorous Components in Anaerobically or Aerobically Digested Liquid Swine Manure, Bull Chem Soc Japan, vol 5(1), p 114-117, 1979
143. van Velsen, AFM, Anaerobic Digestion of Piggery Waste. 1. The Influence of Detention Time and Manure Concentration, Neth J Agric Sci, vol 25, p 151-169, 1977
144. Corke, CT, Bunce, NJ, Beaumont, AL and Merrick, RL, Identification of Offensive Odor Compounds from Potato Processing Plant Waste Effluent Irrigation Fields, J Agric & Food Chem, vol 27(3), p 646-647, 1979
145. Monsanto Technical Bulletin IC/DP-501, Monsanto Phosphoric Acid Handling, Storage Procedures and Precautions, Monsanto, St Louis, 1981

## APPENDIX A: DISCUSSION OF SAMPLE COLLECTION TECHNIQUES

Chemical analyses of water deposited into a plastic bag, plastic cup and vial were performed using the gas chromatograph and mass spectrometer apparatus described in reference 13. All containers were new and unused. The chromatograms showed five large peaks in the plastic bag wash, all identified as grease. The chromatograms of the water that rinsed the plastic cup and vial also showed many peaks. Impurities in the containers for some of the samples added to the level of background peaks so that there was a major interference with the sample analysis.

Several compounds were identified in the water rinse of a rubber catheter, the results of which are shown in table A-1. This level of contamination suggests that urine samples intended for chemical analysis should not be obtained with a catheter made of this material. A teflon catheter, if available and properly cleaned, may be acceptable.

In the future, it is recommended that all glass containers with teflon or foil lid liners be used. All glassware should be cleaned by hand, and after washing with HPLC-Grade solvent (acetone, hexane), the container should be given a final wash in dilute HF (1-3%). It is advantageous that a reasonable quantity of the cleaned containers be obtained from the laboratory for sample collection. This approach would save the effort needed in confirming that all solvents including the water were organic free.

PREVIOUS PAGE  
IS BLANK 

ACIDIC FRACTION	ESTIMATED RELATIVE ABUNDANCE	NEUTRAL FRACTION	ESTIMATED RELATIVE ABUNDANCE
	SE-30		OV-101
	OV-101		
Palmitic Acid	0.30	Palmitic Acid	1.0
Stearic Acid	1.0	Stearic Acid	0.57
Myristic Acid	0.063		
Inositol	0.038		

Table A-1. Principal chemical components in the acidic and neutral fractions of a water extract of a rubber catheter. The estimated relative abundances are indicated with those of the most abundant component for each chromatographic column type, equal to one.

**END**

**FILMED**

*2-86*

**DTIC**