Table V Plasma vitellogenin levels (μg ml^{-1}) in male rainbow trout injected with estradiol-17β (E) and 17α-ethynylestradiol (EE). Means of 4 fish. Temperature 8.5°C

Dose (μg kg^{-1})		Days after injection					
		1	5	10	15	20	25
1	E	<10	15	18	22	13	10
	EE	**<10**	**39**	**87**	**155**	**66**	**88**
100	E	<10	52	61	84	63	75
	EE	**<10**	**130**	**172**	**480**	**221**	**184**
500	E	<10	82	92	173	108	126
	EE	**<10**	**261**	**3 750**	**15 050**	**13 110**	**90 750**
1 000	E	<10	256	240	5 690	6 950	4 295
	EE	**<10**	**158**	**3 360**	**13 250**	**16 000**	**20 300**

Controls for each steroid were < 10 μg ml^{-1} on each sampling day

in the pill is 17 α–ethynylestradiol but its 2-methyl ester (Mestranol) is sometimes used. The main metabolites of these artificial steroids are the α- and β-glucuronides.

In a preliminary trial to assess whether ethynylestradiol could induce vitellogenesis in trout, male fish were given a single intramuscular injection of varying doses of ethynylestradiol and, for comparison, similar doses of the natural steroid estradiol-17β. Sham injected controls were run for each group of fish and individuals were identified by tagging. The results in Table V show that the artificial steroid 17 α-ethynylestradiol can induce vitellogenesis in trout and that it is very much more potent than estradiol-17β.

In a second trial, male fish were exposed by immersion to ethynylestradiol, Mestranol and the α- and β-glucuronides at a concentration of 25 ng l^{-1}. Blood samples from 10 individually identified fish at each treatment were taken two days after the start of treatment and daily thereafter, up to the sixth day. All steroids other than ethynylestradiol were ineffective by comparison with untreated controls. The effect of ethynylestradiol is shown in Figure 1. A logarithmic increase in serum vitellogenin occurred over the 6 day period to reach levels approaching those commonly found in mature females.

Finally, within this series, male trout and immature carp were exposed by immersion to different concentrations of ethynylestradiol for a preliminary evaluation of dose response. At 9.5°C (Table VI), carp responded much less strongly than trout but significant enhancement was apparent at steroid concentrations at and above 10 ng l^{-1}. In the parallel trout group, the lowest steroid concentration of 1 ng l^{-1} appeared ineffective but the samples used in the vitellogenin assay were over-diluted and below the sensitivity of the assay. In a later series of experiments conducted at a higher temperature (Table VII), significantly enhanced levels of vitellogenin were found with all treatments. The responses at 10 ng l^{-1} in Tables VI and VII are within the range of observations at STWs as shown in Table II.

DISCUSSION

This paper confirms that estrogenic substances are present in the effluent of STWs. Its preliminary nature and the difficulty of conducting repeatable, replicated trials in field conditions precluded the application of full scientific rigour. The resultant data

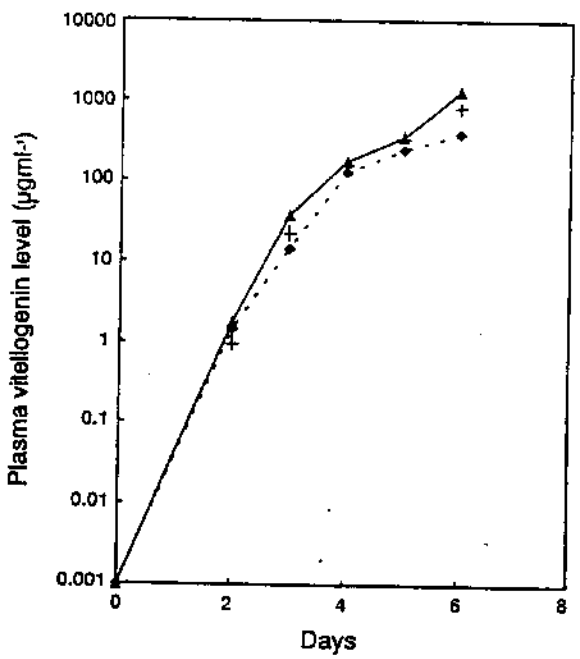

Figure 1 Effect on plasma vitellogenin levels ($\mu g\ ml^{-1}$) of immersion of male trout in 25 ng l^{-1} of ethynylestradiol for 6 days. + mean of 10 fish; ▲ individual fish showing greatest response over 6 days; ◆ individual fish showing least response over 6 days.

Table VI Vitellogenin levels ($\mu g\ ml^{-1}$) in male rainbow trout and immature carp exposed to 17α-ethynylestradiol by immersion for 10 days (temperature 9.5°C). Mean and SEM of 10 fish in each group

Dose (ng l^{-1})	Plasma vitellogenin ($\mu g\ ml^{-1}$) mean ± SEM	
	Carp	Trout
Control	<0.01	<1.0
1	<0.01	<1.0#
10	0.15 ± 0.08*	630 ± 140**
25	0.84 ± 0.26**	4970 ± 73**
50	216 ± 26**	11200 ± 800**

Statistical significance of response compared to controls
*P<0.05
**P<0.001
#Samples overdiluted for assay

set, although imperfect, indisputably demonstrates the nationwide distribution of estrogens in STW effluents; fuller and more detailed studies are currently in progress.

Placing rainbow trout in the effluent of sewage-treatment works caused a rapid and very pronounced increase in their plasma vitellogenin concentrations. It is

Table VII Vitellogenin levels in male trout after 10-day exposure to 17α-ethynylestradiol by immersion (temperature 16.5°C)

Dose (ng l^{-1})	n	Vitellogenin (μg ml^{-1})	Range (μg ml^{-1})
Control	10	<0.01	All<0.01
0.1	10	0.06 ± 0	0.02–0.01*
0.5	8	9.71 ± 5.03	0.34–46**
1.0	10	149 ± 112	0.18–1150**
10.0	7	37400 ± 4130	16500–48000**

Statistical significance of response compared to control
*P<0.05
**P<0.001

unlikely that this marked response was non-specific, because no effect was observed in fish placed in the effluent of a major trout farm and, in any case, stress causes a decrease in the plasma vitellogenin concentration (Carragher *et al.*, 1989; Campbell *et al.*, 1993). It is well established that the synthesis of vitellogenin in trout is under the control of estrogens; in female trout, estradiol-17β is the naturally occurring estrogen primarily responsible for stimulating vitellogenesis during sexual maturation (van Bohemen *et al.*, 1982; Scott and Sumpter, 1983). In a comprehensive series of experiments, Bromage and colleagues injected a large number of different steroid hormones into rainbow trout, and assessed their ability to stimulate vitellogenin synthesis (reviewed in Bromage and Cumaranatunga, 1988); they found that estrogens were very much more effective than other steroids. LeGuellec *et al.* (1988) describe primary and secondary stimulation of vitellogenesis in male trout by injection of estradiol-17β. Thus, it is generally accepted that vitellogenin synthesis in trout (and other oviparous vertebrates) is primarily under the control of estrogens, and can be induced by exogenous estrogens even in male fish. Hence, the enhanced synthesis of vitellogenin in the trout maintained in sewage effluent must have been due to the presence of an estrogenic substance (or substances) present in the effluent.

The nature of the estrogenic compound(s) in effluent is not known. The *a priori* hypothesis was that ethynylestradiol, originating from use of the contraceptive pill, was implicated. Laboratory tests of the potency of this and other steroids were conducted. Attempts were also made to measure contaminant levels in sewage effluents by GC-MS and radioimmunoassay. Both of these attempts were unsuccessful and technical problems preclude unequivocal interpretation of the data (see also Aherne *et al.*, 1985). Notwithstanding this, the assessment of the efficacy of ethynylestradiol by injection or by immersion has shown it to be an extremely potent inducer of vitellogenesis, far exceeding the effect of estradiol-17β, the natural estrogen implicated in vitellogenesis. Concentrations of 10 ng l^{-1} in the water generated increases in plasma vitellogenin of a similar magnitude to those observed in the nationwide survey of STWs. Concentrations as low as 0.1 ng l^{-1} also caused a significant rise in plasma vitellogenin and this has been confirmed by more recent studies (Sheahan *et al.*, 1993). This places the artificial estrogen ethynylestradiol amongst the most potent of biologically active molecules.

Although various phytoestrogens and pesticides with low estrogenic potencies could contribute to an overall estrogenic effect, it has always seemed unlikely that they could be present in effluents at sufficiently high concentrations to be serious contributors to the phenomenon. Recently, however, a further possibility was suggested (A.J. Dobbs, personal communication, 1991), namely nonylphenols, which

can be present in sewage systems and which have been shown to be estrogenic in an estrogen-sensitive human cell assay (Soto *et al.*, 1991). Amongst the group of related alkylphenol-ethoxylates (APE), the nonylphenols are major degradation products of surfactants and detergents and can be present in large amounts in sewage. In a survey of STWs, Waldock and Thain (1986) concluded that concentrations of nonylphenols were generally low (<2 to 21 ng l^{-1}) in effluents but were present at higher levels in sludges (0.3 mg kg^{-1} dry weight). Levels (<25 to 314 ng l^{-1}) which were also higher than in STW effluent were observed in water samples taken from the mouth of the River Mersey (MAFF, 1991). Detailed assessment of the possible significance of APE is being conducted and will be reported elsewhere. The present position appears to be that the choice of hypothesis to account for the estrogenic activity in STW effluent lies between (a) ethynylestradiol, highly potent at nanogram per litre concentrations, but not yet detectable chemically, and (b) APE, which may be 4 or more orders of magnitude less potent than ethynylestradiol, but is more readily demonstrable in sewage systems.

The full implication of the discovery that effluents from STWs are estrogenic is not yet clear but studies on the reproductive impact for fish are underway. The demonstration that the estrogenic effect is dissipated downstream, and the largely negative results from abstraction points and potable water supplies do limit concern on public health grounds but wider study is desirable here too. Going back to the original observation of hermaphrodite fish, this is unlikely to be a consequence of the estrogenic effect of effluent. Changes to the primary sexual characters, the gonads, of fish occur only when estrogenic treatments are made at an early stage in the life of fish fry (Yamamoto, 1969). The fish in the STW lagoons were not hatched there, but introduced as mature fish.

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ATTACHMENT #12

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CHRONIC AND SUBLETHAL TOXICITIES OF SURFACTANTS TO AQUATIC ANIMALS: A REVIEW AND RISK ASSESSMENT

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Abstract—Surfactants are one of the major components (10–18%) of detergent and household cleaning products and are used in high volumes. Several are commonly found in natural waters and consequently, their impact on the environment has been, and continues to be, discussed in the U.S.A., Western Europe and Japan. The chronic and sublethal toxicities of commercially important surfactants to aquatic animal life have not been summarized in the available scientific literature. Based on the summary provided here scientific understanding of the chronic and sublethal toxicities of cationic surfactants is less than that for the other surfactant groups. Chronic toxicity of anionic and nonionic surfactants occurs at concentrations usually greater than 0.1 mg/l. Effects of these same surfactants on several behavioral and physiological parameters range from 0.002 to 40.0 mg/l. The available toxicity data base is largely comprised of laboratory-derived toxicity data for a few surfactants, predominantly LAS, and single freshwater planktonic species such as *Daphnia magna* and the fathead minnow and a benthic midge. Community effect levels have been reported only for linear alkylbenzene sulfonate (LAS) and effects on single freshwater and saltwater test species and on natural biotic communities are largely unknown for many commercially important surfactants. Based on a comparison of the reported chronic toxicity data and measured environmental levels in rivers, the aquatic safety of the anionic LAS is indicated, more so than for any other surfactant. Safety assessments for other major surfactants in saltwater and freshwater should be considered preliminary and limited until validated with corresponding exposure measurements and additional laboratory and field-derived chronic toxicity data for animal test species.

Key words—surfactants, chronic toxicity, review, risk assessment, environmental concentrations

INTRODUCTION

Surfactants are synthetic organic chemicals used in high volumes in detergents, personal care and household cleaning products. These compounds usually comprise 10–18% of granular and liquid detergents and are the largest ingredient of the 20–25 compounds used in these products (Höglund, 1976; Richtler and Knaut, 1988). Surfactants are used also by the oil, textile, food and mining industries. Although there are many surfactant types, linear alkylbenzene sulfonates, alkyl sulfates, alkyl ether sulfates, alkyl ethoxylates, alkyl phenol ethoxylates and quaternary ammonium halide compounds are common in commercial detergent applications (Richtler and Knaut, 1988). Approximately 15 million tons of soap and synthetic surfactants were used worldwide in 1987 (Berth and Jeschke, 1989). Surfactants, due to their widespread use, have been measured at various concentrations in river water, drinking water, sediments and sludge-amended soils (Sivak *et al.*, 1982; McEnvoy and Giger, 1985; DeHenau *et al.*, 1986; Giger *et al.*, 1987; Brunner *et al.*, 1988; Ventura *et al.*, 1989). As a result of their presence primarily in river water, the environmental effects and fate of anionic and cationic surfactants have been discussed at various international seminars and symposia (German Chemical Society, 1982; Richtler and Knaut, 1988; Ruchay, 1989) and have been reviewed by regulatory agencies primarily in Western Europe and Japan where dilution of the receiving water and sewage treatment are less than in the U.S.A.

The toxicities of surfactants to aquatic life have been summarized previously in the scientific literature (Abel, 1974; A. D. Little Co., 1977, 1981; Koskova and Kozlovskaya, 1978; Margaritis and Creese, 1979; Sivak *et al.*, 1982; Lewis and Suprenant, 1983; Lewis and Wee, 1983; Cooper, 1988). Environmental assessments based on these reviews, however, are outdated considering the constant development of new surfactants and reformulation of existing surfactant components in detergent products. In addition, the previously summarized data typically are limited to acute toxicity values for a few surfactants, primarily the anionic and, to a lesser extent, nonionic forms. Many reviews contain few or no chronic and sublethal toxicity data while others do not consider cationic surfactants.

A comprehensive and current summary of the chronic and sublethal effects of surfactants to aquatic animals is needed, since contemporary environmental safety assessments, particularly the toxicity assessment phase, are based on chronic toxicity information. In addition, the need to evaluate the role of sublethal effects in the safety assessment process has

been identified as one of the key future research priorities in the environmental risk assessment process (Society of Environmental Toxicology and Chemistry, 1987). The first phase in gaining an insight into this role is to understand the data base. Therefore, a summary of the chronic and sublethal toxic concentrations for surfactants would be helpful in consolidating the data base, providing an overview of their potential environmental impact based on chronic effects and indicating the priority for future research. This review represents a comprehensive summary of these effects for commercially important surfactants and freshwater and saltwater animal life.

METHODS

Structures of several representative surfactants for which chronic toxicity data have been reported appear in Fig. 1. The test methods used to determine the toxicity of these and other surfactants have not been consistent; the test species, test durations, effect parameters, the test compound and analytical confirmation of the test concentrations are several experimental variables that have differed. The analytical verification of the test concentrations, an important consideration, was not a common occurrence in the reviewed studies. Therefore, the results summarized in the tables, unless noted, are based on nominal concentrations. Chronic toxicity tests typically include life cycle, partial life cycle and early life stage tests (Stephan *et al.*, 1985). In many cases the types of studies reviewed here did not represent these categories and consequently in a strict sense do not represent chronic toxicity data as commonly accepted by the scientific community. However, for simplicity, data generated in tests exceeding normal acute test durations of 48 h for invertebrates and 96 h for fish were included as "chronic toxicity" data.

Generic name	Structure
Linear alkylbenzene sulfonate (LAS)	$CH_3-(CH_2)_x-CH_3$  SO_3Na ; $x = 7\text{-}14$
Linear alkylethoxylate (AE)	$CH_3-(CH_2)_x-(C_2H_4O)_y-H$; $x = 7\text{-}19$, $y = 0\text{-}12$
Cetyl trimethyl ammonium bromide (CTAB)	$CH_3(CH_2)_{14}CH_2-N^+(CH_3)_2[Br]^-$
Ditallow dimethyl ammonium chloride (DTDMAC)	$CH_3(CH_2)_n-N^+(CH_3)-(CH_2)_nCH_3\ [Cl]^-$; $n = 15,17$

Fig. 1. Structures of representative surfactants commonly used in commercial detergent and softener products.

The amount of detail possible in a summary paper of this type is limited. Additional detail concerning experimental technique and, in some cases, additional toxicity data can be found in the reviewed papers.

RESULTS

Chronic Toxicity

Invertebrates

Daphnia magna has been the most common test species (Table 1). The effect concentrations for this species and LAS (linear alkylbenzene sulfonate), the predominant test compound, have ranged from 0.005 to > 10.0 mg/l; however, the more typically reported chronic effect concentrations exceed 0.1 mg/l (Fig. 2). Values less than 0.1 mg/l are few and the 0.005 mg/l effect value for *D. magna* reported by Lal *et al.* (1984) should be considered an outlier. LAS has been used for 25 years in granular and liquid detergent products, shampoos, soaps, shaving creams and industrial cleaners. Based on data from Taylor (1985), the first effect concentration range (geometric mean of NOEC and LOEC) for six 21-d chronic toxicity tests conducted with *D. magna* and $C_{11.8}$ LAS, an approximate alkyl chain length blend commonly used in commercial products, was 1.7–3.4 mg/l. The no observed effect concentrations for these studies ranged from 1.3 to 3.3 mg/l and the 21-d LC_{50} values, 2.2 to 4.7 mg/l. Kimerle (1989) reported NOEC values for *D. magna* and several LAS homologues that ranged from 0.1 mg/l (C_{14} homologue) to 9.8 mg/l (C_{10} homologue). The NOEC value for a $C_{11.7}$ LAS blend and *Ceriodaphnia* was 3.0 mg/l. Masters *et al.* (1991) reported that the first effect concentrations for *Ceriodaphnia* and $C_{11.8}$ LAS were <0.32 and 0.89 mg/l.

The effect concentrations for LAS and other invertebrate species are similar to those observed for daphnids. Effect concentrations were between 0.2 and 0.4 mg/l for *Gammarus* exposed to LAS (Arthur, 1970). Pittinger *et al.* (1989) reported that the NOEC for the midge was 319 μg/g and the LOEC (lowest observed effect concentration) was 993 μg/g based on sediment-adsorbed LAS concentrations. Likewise, Bressan *et al.* (1989), reported the relatively low toxic nature of sediment-adsorbed LAS to other freshwater and marine benthic organisms.

The toxicities of other anionic surfactants, based on limited data appears to be similar to that for LAS (Table 1). For example, first-effect concentrations for alkyl sulfate (AS) compounds were reported between 0.25 and 1.46 mg/l for flatworms and oyster and clam larvae (Hidu, 1965; Patzner and Adam, 1979) and a NOEC of 0.27 mg/l was reported for *D. magna* and an alkyl ethoxy sulfate (Maki, 1979a).

The chronic effects of several nonionic alkyl ethoxylates (AE) and the cationic ditallow dimethyl ammonium chloride (DTDMAC) to *D. magna* occur between 0.1 to 1.0 mg/l. Maki (1979a), for example,

Table 1. Reported chronic toxicities of surfactants to invertebrates

Surfactants	First effect concentration (mg/l)	Test species	Test duration	Effect	Reference
Anionic					
$C_{11.8}$LAS[1]	1.7–3.4[2]	*Daphnia magna*	21 d	Survival Reproduction	Taylor (1985)
LAS	>10.0 (NOEC)	*Daphnia magna*	21 d	Reproduction	Canton and Slooff (1982)
$C_{11.8}$ LAS	1.18 (NOEC)*	*Daphnia magna*	21 d	Reproduction	Maki (1979a)
C_{12} LAS	0.57 (NOEC)*				
AES[3]	0.27 (NOEC)*				
AS[4]	0.25	Flatworms:			
		Dugesia gonocephala	30 d	Regeneration	Patzner and Adam (1979)
		Notoplana humilis			
LAS	0.2–0.4*	*Gammarus pseudolimnaeus*	6–15 wk	Growth, reproduction	Arthur (1970)
	0.4–1.0*	*Campeloma decisum* (snail)			
	>4.4*	*Physa integra* (snail)			
LAS	0.05–0.10	Oyster (*Crassostrea virginica*)	10 d	Larval growth, egg development	Calabrese and Davis (1967)
		Ceriodaphnia dubia	7 d	Reproduction	Masters *et al.* (1991)
$C_{11.8}$ LAS	<0.32, 0.89	*D. magna*	ND	Reproduction	Kimerle (1989)
LAS (C_{10}–C_{14} homologues)	0.1–9.8 (NOEC range)				
$C_{11.7}$ LAS	3.0 (NOEC)	*Ceriodaphnia* sp.	ND	Reproduction	Kimerle (1989)
$C_{12.7}$ LAS	0.04 (NOEC)	Mysid shrimp (*Mysidopsis bahia*)	ND	ND	Kimerle (1989)
$C_{11.8}$ LAS	0.4 (NOEC)	Clams (*Mercenaria mercenaria*)	14 d	Larval growth and development	Hidu (1965)
ABS[5]	0.55–5.8	Oysters (*C. virginica*)			
	0.14–1.63	*M. mercenaria*	14	Larval growth and development	Hidu (1965)
AS	0.47–1.46	*C. virginica*			
	0.37–1.46	Midge (*Chironomus riparius*)	24 d	Emergence	Pittinger *et al.* (1989)
$C_{11.8}$ LAS	993 SC[6,8]				
	15.2 IW[7]				
	1.69 OW[8]				
	3.72 NS[9]				
LAS	0.05	Mussel (*Mytilus edulis*)	10 d	Fertilization, larval growth	Granmo (1972)
Nonionic					
C_{12-13} $AE_{6.5}$[10]	0.24 (NOEC)*	*D. magna*	21 d	Reproduction	Maki (1979a)
C_{14-15} AE_7	0.24 (NOEC)*				
C_{14-15} AE_7	0.17, 0.70	*C. dubia*	7 d	Reproduction	Masters *et al.* (1991)
C_{14-15} AE_7	1.0	*D. magna*	30 d	Reproduction	Sherberbo (1980)
Lauror-9	0.25–0.50	*D. gonocephala*	30 d	Regeneration	Patzner and Adam (1979)
C_{12-15} AE_{10}		*N. humilis*			
	<0.1–20	*M. edulis*	5 mth	Fertilization, spawning	Granmo and Jorgensen (1975)
TAE_{10}					
	1.75–2.5	*M. mercenaria*	14 d	Larval growth and development	Hidu (1965)
Alkyl polyether alcohol	1.6–2.5	*C. virginica*	14 d	Larval growth and development	Hidu (1965)
Iso-octyl phenoxy polyethoxy ethanol	0.77–2.5	*M. mercenaria*			
	0.86–1.0	*C. virginica*	14 d	Larval growth and development	Hidu (1965)
APE[11]	2.4	*M. edulis*			
Cationic					
TMAC[12]	0.065 (NOEC)*	*D. magna*	ND	ND	Pittinger *et al.* (1989)
TMAC	0.17, 0.35	*C. dubia*	7 d	Reproduction	Masters *et al.* (1991)
DTDMAC[13]	0.38–0.76*	*D. magna*	21 d	Reproduction	Lewis and Wee (1983)
DSDMAC[14]	2708 SC[6,8]	*C. riparius*	24 d	Emergence	Pittinger *et al.* (1989)
	0.18 IW[7]				
	0.41 OW[8]				
	1.02 NS[9]				
TMAC	>3084 SC[6,8]	*C. riparius*	24 d	Emergence	Pittinger *et al.* (1989)
	>2.3 IW[7]				
	>0.9 OW[8]				
	0.62 NS[9]				
Lauryl pyridinium chloride	0.009–0.05	*M. mercenaria*	14 d	Larval growth and development	Hidu (1965)
	0.05–0.09	*C. virginica*			
Ethyl dimethyl benzyl ammonium chloride	0.25–1.27	*M. mercenaria*	14 d	Larval growth and development	Hidu (1965)
	0.10–0.49	*C. virginica*			

[1]LAS = linear alkylbenzene sulfonate. [2]Range of first effect levels for six studies. [3]AES = alkyl ethoxy sulfate. [4]AS = alkyl sulfate. [5]ABS = alkylbenzene sulfonate. [6]Sediment concentration LOEC in μg/g. [7]Interstitial water concentration LOEC in mg/l. [8]Overlying water concentration LOEC in mg/l. [9]LOEC in study with no sediment. [10]AE = alkyl ethoxylate. [11]APE = alkylphenol ethoxylate. [12]TMAC = dodecyl trimethyl ammonium chloride. [13]DTDMAC = ditallow dimethyl ammonium chloride. [14]DSDMAC = distearyl dimethyl ammonium chloride. NOEC = no observed effect concentration. *Value based on measured concentrations. ND = no data.

reported a NOEC of 0.24 mg/l for two nonionic alkyl ethoxylates. The first effect concentrations of an AE for *Ceriodaphnia* were 0.17 and 0.70 mg/l in tests of 7 days duration (Masters *et al.*, 1991). Lewis and Wee (1983) reported that the first effect level for DTDMAC was between 0.38 (NOEC) and 0.76 mg/l

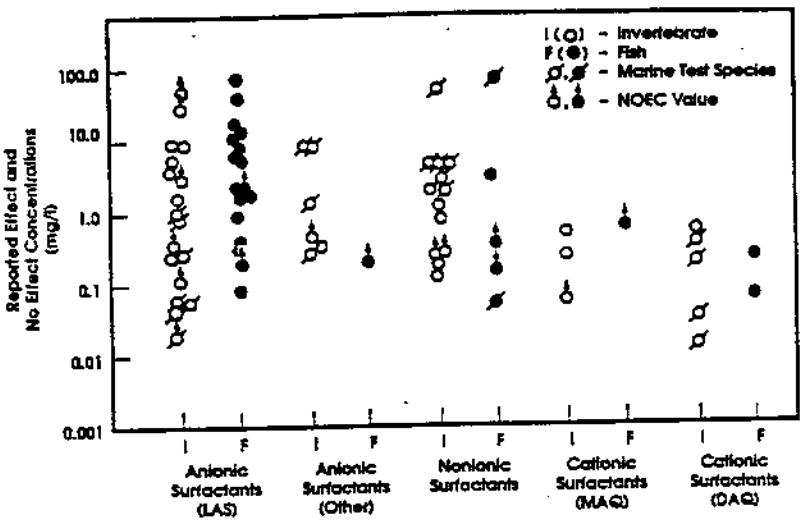

Fig. 2. Reported effect and no effect levels for surfactants. MAQ = monoalkyl quaternary ammonium salts; DAQ = dialkyl quaternary ammonium salts.

(LOEC) for a study conducted in river water. DTDMAC is used primarily as softening agent in fabric softeners and as an anti-static agent on drier sheets. This same cationic compound adsorbed to sediment was toxic to early life stages of midge only at very high level concentrations (Pittinger *et al.*, 1989). Lee (1986) found that the safety margin for the midge and sediment-bound DTDMAC ranged from 17 to 105 based on two partial life-cycle tests.

A NOEC of 0.065 mg/l has been reported for *D. magna* and a monoalkyl quaternary ammonium compound, TMAC (Pittinger *et al.*, 1989). The first effect concentrations derived from two tests using a similar compound for *Ceriodaphnia* were 0.17 and 0.35 mg/l (Masters *et al.*, 1991).

The chronic effect concentrations for surfactants and marine organisms have been reported primarily for clams, oysters and mussels. Effects of LAS on oysters and mussels based on changes in fertilization, egg development and larval growth have occurred at concentrations generally exceeding 0.025 mg/l (Calabrese and Davis, 1967; Granmo and Jorgensen, 1975). The first effect levels for several nonionic compounds on clam and oyster larvae were between 0.8 and 2.5 mg/l (Hidu, 1965) and at concentrations less than 0.1 mg/l for an alkyl ethoxylate (Granmo and Jorgensen, 1975). Threshold values of 0.010 and 0.050 mg/l LAS have been reported for oysters and sponges (Berth *et al.*, 1988). The NOEC values for the mysid shrimp and two LAS blends, $C_{11.4}$ and $C_{13.1}$, were 0.4 and 0.04 mg/l, respectively (Kimerle, 1989). Hidu (1965) reported the effects of two cationic compounds on clam and oyster larvae and the lowest first effect concentration was 0.0085 mg/l. Overall, the cationic surfactants were the most toxic of the surfactants tested in that study.

Fish

The reported chronic toxicities for surfactants and fish are based largely on the response of fathead minnows to various blends and homologues of the anionic LAS (Table 2; Fig. 2). The first effect levels for LAS exceed 0.1 mg/l in most cases for the fathead minnow (Macek and Sleight, 1977; Holman and Macek, 1980), and for other fish species (Vailati *et al.*, 1975; Canton and Slooff, 1982; McKim *et al.*, 1975; Chattopadhyay and Konar, 1986a). Holman and Macek (1980) for example, reported NOEC values of 0.11–5.1 mg/l and LOEC values of 0.25–8.4 mg/l for fathead minnows in life cycle and early life stage tests using several LAS blends. The NOEC values for C_{13} LAS and $C_{11.8}$ LAS and the fathead minnow were 0.15 and 0.90 mg/l, respectively (Maki, 1979a). The greater toxicity of the higher alkyl chainlength LAS blends observed by Maki (1979a) has been reported elsewhere (Kimerle and Swisher, 1977; Macek and Sleight, 1977; Holman and Macek, 1980). The first effect concentration of a C_{14} LAS homologue was between 0.05 and 0.10 mg/l for the fathead minnow relative to 14.0–28.0 mg/l for a C_{10} LAS homologue (Macek and Sleight, 1977).

Relatively few chronic toxicity values have been reported for nonionic and cationic surfactants and fish (Table 2; Fig. 2). The NOEC values for two nonionic alkyl ethoxylates were 0.18 and 0.32 mg/l, respectively (Maki, 1979a) whereas a nonionic oil dispersant was toxic at 0.05 mg/l to one marine flatfish but not another (Yasunaga, 1976). Chattopadhyay and Konar (1986b) reported that fecundity of *Tilapia* was reduced after exposure to 3.98 mg/l nonionic surfactant. Only two toxicity reports were found for cationic surfactants, Lewis and Wee (1983) reported that the first effect concentration for

Table 2. Reported chronic toxicities of surfactants to fish

Surfactant	First effect concentration (mg/l)	Test species	Test duration	Effect	Reference
Anionic					
C_{10} LAS	0.90 (NOEC)*	Fathead minnow	28 d	Hatching, growth, larval survival	Maki (1979a)
C_{11} LAS	0.15 (NOEC)*				
AES	0.10 (NOEC)*				
C_{11} LAS	5.1–8.4*	Fathead minnow	Complete life cycle, partial life cycle	Hatching, growth, larval survival	Holman and Macek (1980)
C_{12} LAS	0.48–0.49*				
C_{13} LAS	0.11–0.25*	Fathead minnow	28 wk	Survival	Pickering and Thatcher (1970)
LAS	0.63–1.2†				
C_{10} LAS	14.0–28.0	Fathead minnow	28 d	Survival, hatching	Macek and Sleight (1977)
C_{11} LAS	7.2–14.5				
C_{12} LAS	1.08–2.45				
C_{13} LAS	0.12–0.28				
C_{14} LAS	0.05–0.10				
LAS	3.2 (NOEC)	*Poecilia reticulata*	28 d	Immobility	Canton and Slooff (1982)
LAS	0.05–0.50	Marine flatfish (*Limanda yokohamae, Paralichrys olivaceus*)	30 d	Hatching	Yasunaga (1976)
LAS	2.0–5.0	Fathead minnow	30 d	—	Swisher *et al.* (1978)
LAS	0.25–1.10	*Tilapia massambica*	90 d	Fecundity, maturity	Chattopadhyay Konar (1986a)
LAS	4–10	Bluegill	6 d	Fertilization, hatching	Hokanson and Smith (1971)
LAS	0.5–1.1*	Fathead minnow	30 d	Standing crop	McKim *et al.* (1975)
	<0.3*	White sucker			
	0.5–1.2*	Northern pike			
	2.3–5.8*	Smallmouth bass			
Nonionic					
C_{12-13} AE	0.32 (NOEC)*	Fathead minnow	28 d	Growth, hatching, larval survival	Maki (1979a)
C_{12-15} AE	0.18 (NOEC)*				
Oil dispersant	10–50	*Limanda yokohamae, Paralichrys olivaceus*	10 d	Hatching	Yasunaga (1976)
Oleyl-cetyl alcohol Ethylene oxide condensate	0.05 <3.98	*Tilapia massambica*	90 d	Fecundity, maturity	Chattopadhyah and Konar (1986b)
Cationic					
DTDMAC	0.05–0.09†*	Fathead minnow	28 d	Growth, hatching	Lewis and Wee (1983)
	0.23–0.45‡*				
TMAC	0.46 (NOEC)*	Fathead minnow	ND	ND	Pittinger *et al.* (1989)

†Test conducted in laboratory water.
‡Test conducted in river water.
*Value based on measured test concentrations.
ND = no data.

DTDMAC was between 0.05 (NOEC) and 0.09 mg/l (LOEC) for fathead minnows exposed in laboratory water and between 0.23 (NOEC) and 0.45 mg/l (LOEC) in river water. The NOEC for C_{12} trimethyl ammonium chloride and the fathead minnow was 0.46 mg/l (Pittinger *et al.*, 1989).

Sublethal Toxicity

Physiological responses

The majority of reports describe the effects of anionic surfactants on several physiological processes of fish during exposures of 15 min to 30 days (Table 3). Effects on olfaction, respiration and gill physiology were more frequently monitored than other parameters and effects occurred at concentrations that exceed 0.1 mg/l in most cases. For example, changes in adrenergic control mechanisms and vasodilation in salmon gills were noted at LAS concentrations of 0.6 mg/l or greater (Bolis and Rankin, 1978, 1980). The respiratory rate of bluegills was first altered at concentrations ranging from 0.39 to 2.20 mg/l for several anionic surfactants (Maki, 1979b). The low effect concentrations of 0.005 and 0.015 mg/l were reported for LAS based on changes in gill and skin morphology after 30 days of exposure (Misra *et al.*, 1985, 1987).

Sutterlin *et al.* (1971), in a comprehensive study, tested many surfactants for their stimulatory and blocking effectiveness on the olfactory epithelium of Atlantic salmon. Blocking effects were noted at 1 mg/l for several of the cationic surfactants and the anionic alkylbenzene sulfonate. No blocking effect was noted for the nonionic surfactants. Overall, the effects were reversible in many cases. Maciorowski *et al.* (1977) also reported that the effects of an anionic surfactant on intestinal damage to clams was reversible. The no observed effect concentrations based on the respiratory rate of bluegill were 0.54 and > 1.56 mg/l for two alkyl ethoxylates (Maki, 1979b).

The physiological effect concentrations of anionic surfactants on species other than fish have ranged

from 0.015 to 3.0 mg/l (Table 3). Moffett and Grosch (1967), for example, reported that 1–3 mg/l LAS caused developmental abnormalities in several marine invertebrates whereas 0.015 mg/l ABS reduced calcium uptake in a snail after 72 h exposure (Misra *et al.*, 1984).

Behavioral responses

The avoidance reaction by fish has been one of the more commonly monitored effect parameters in behavioral studies with surfactants. Avoidance of several anionic surfactants by a variety of fish species has been observed at concentrations ranging from 0.002 to 0.40 mg/l (Table 4). The concentration resulting in a 65% avoidance ratio by the Ayu for several anionic surfactants was 0.002–0.011 mg/l (Tatuskawa and Hidaka, 1978) whereas avoidance reactions of another fish species, the Medaka, for similar compounds ranged from 0.007 to 0.027 mg/l (Hidaka *et al.*, 1984). Other responses such as swimming activity and feeding behavior are affected at higher concentrations. The effects of LAS on these characteristics for trout, goldfish, cod and carp have occurred at concentrations between 0.2 and 5.0 mg/l (Marchetti, 1968; Swedmark *et al.*, 1976; Sabourau and Lesel, 1977; Walzak *et al.*, 1983). For example, the swimming activity of trout was altered at 0.2–0.4 mg/l LAS (Sabourau and Lesel, 1977) and that of carp at 5 mg/l after 125 d exposure (Walczak *et al.*, 1983).

The reported behavioral effect concentrations for nonionic surfactants have ranged from 0.002 to 40.0 mg/l (Table 4). Höglund (1976) reported that cod avoided a tallow alkyl ethoxylate and a nonylphenol compound at 0.002 mg/l. The avoidance responses however, were erratic in many cases. Swedmark *et al.* (1971), in a comprehensive study, reported the effects of a variety of surfactants including several nonionic compounds on several characteristics of marine fish and invertebrates. Effect levels exceeded 0.5 mg/l in all cases based on changes in swimming activity, shell closures, byssal activity, locomotion and burrowing. Byssal activity and growth of mussels were affected by 0.056 mg/l of a nonylphenolic compound (Granmo *et al.*, 1989). The behavioral effects of cationic surfactants on aquatic life have not been reported.

Table 3. Sublethal responses (physiological/histopathological) to surfactants as reported in the literature

Surfactants	Effect concentration (mg/l)	Test species	Effect	Reference
Anionic				
$C_{11.8}$ LAS	2.2	Bluegill	Respiration	Maki (1979b)
C_{13} LAS	<0.39			
AES	0.39			
ABS	0.5	Yellow bullhead	Chemoreception of taste buds	Bardach *et al.* (1965)
LAS				
ABS	1.0	Atlantic salmon	Olfaction	Sutterlin *et al.* (1971)
SLS[1]	0.1	Whitefish	Depressed olfactory response	Hara and Thompson (1978)
NaC_{12} AS				
LAS	1.5–2.5	Catfish	Separation of gill lamellae	Zaccone *et al.* (1985)
LAS	1.0	Brown trout	Noradrenaline response in gills	Bolis and Rankin (1980)
		European eel		
LAS	0.6–0.3	Pacific salmon	Gill vasodilation	Bolis and Rankin (1978)
LAS	1.0	Rainbow trout	Skin degeneration	Pohla-Gubo and Adam (1982)
LAS	0.005	*Cirrhina mrigala*	Gill morphology	Misra *et al.* (1985)
LAS	1–3	Sea urchin (*Arbacia*)	Developmental abnormalities	Moffett and Grosch (1967)
		Starfish (*Asterias*)		
		Sponge (*Spicula*)		
		Annelid (*Chaetopteris*)		
		Tunicate (*Malgula*)		
NaC_{12}AS	0.67–1.04	Pacific oyster (*Crassostrea gigas*)	Abnormal development	Cardwell *et al.* (1978)
NaC_{13}AS	28	Sea urchin (3 spp)	Inhibition of micromere formation	Tanaka (1976)
LAS	0.005	*Cirrhina mrigala*	Skin morphology	Misra *et al.* (1987)
SLS	0.61	Snail (*Limnaea peregra*)	Shell dry weight	Tarazona and Nunez (1987)
ABS	0.015	Snail (*Limnaea vulgaris*)	^{45}Calcium uptake	Misra *et al.* (1984)
LTBS[2]	1.0	Clam (*Pisidium casertanum*)	Intestinal damage	Maciorowski *et al.* (1977)
LAS	3.5	Rainbow trout	Gill uptake of cadmium	Pärt *et al.* (1985)
Nonionic				
C_{14-15} AE_3	0.54	Bluegill	Respiration	Maki (1979b)
C_{12-15} AE	>1.56	Bluegill	Olfaction	Sutterlin *et al.* (1971)
Several nonionic surfactants	>10	Atlantic salmon		
Cationic				
Several quaternary ammonium and imidazolinium salts	1.0	Atlantic salmon	Olfaction	Sutterlin *et al.* (1971)

[1]SLS = sodium lauryl sulfate.
[2]LTBS = linear tridecyl benzene sulfonate.

Data overview

Overall, most c
scientific literatur
More specifically,
data base availa
limited to a few c
primarily various
in U.S.A., Japan
and to a lesser e
(467,000 MT) an
data for high-vol
LAS such as the

Surfactant

Anionic
LAS
AS
ABS
LAS
AS
AES
ABS
ABS
ABS
LAS
LAS

LAS

LAS
C_{10-15} LAS

C_{12}-C_{14} LAS

ABS

LAS
ABS
LAS

Nonionic
C_9 APE_{10}

TAE EO(10)
NP EO(10)[4]
NP EO(10)

NP EO(10)

APE[3]
C_9 APE_{10}
NP[3]
TAE-EO(10)
NP-EO(10)
4-NP
Oleyl-cetyl alcohol-ethylene oxide condensate

[3]Nonylphenol etho
[4]Alkylphenol ethox
[5]Nonylphenol.

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DISCUSSION

Data overview

Overall, most of the toxicity data available in the scientific literature is for anionic surfactants (Fig. 2). More specifically, the chronic and sublethal toxicity data base available to the scientific community is limited to a few commercially important surfactants, primarily various blends of LAS (1987 consumption in U.S.A., Japan and Western Europe = 984,000 MT) and to a lesser extent the nonionic alkyl ethoxylates (467,000 MT) and the cationic DTDMAC. Toxicity data for high-volume anionic surfactants other than LAS such as the alkyl sulfates (236,000 MT) and the alkyl ethoxysulfates (350,000 MT) are fewer. The usage values are from Richtler and Knaut (1988). The relative absence of chronic toxicity data for fish is most noticeable, particularly for several major anionic surfactants and cationic dialkyl and monoalkyl quaternary ammonium halide compounds. The U.S.A. and Western Europe consumption of cationics in 1987 was 190,000 and 150,000 MT, respectively (Roes and de Groot, 1988).

The reported chronic toxicity results summarized here are based largely on the response of laboratory cultured single species exposed under controlled laboratory conditions usually for 21 days duration or less. Effects of most surfactants on structural and

Table 4. Sublethal responses (behavioral) to surfactants as reported in the literature

Surfactant	Effect concentration (mg/l)	Test species	Effect	Reference
Anionic				
LAS	0.002	Ayu (*Plecoglossus altivelis*)	Avoidance	Tatuskawa and Hidaka (1978)
AS	0.008			
ABS	0.011	Medaka (*Oryzias latipes*)	Avoidance	Hidaka *et al.* (1984)
LAS	0.014			
AS	0.007			
AES	0.025, 0.027			
ABS	0.014	Rainbow trout	Avoidance	Sprague (1968)
ABS	0.001	Cod (*Gadus morrhua*)	Avoidance	Höglund (1976)
ABS	0.02			
LAS	0.002	Arctic charr (*Salvelinus alpinus*)	Chemoattraction, locomotor activity	Olsén and Höglund (1985)
LAS	0.02	Carp	Swimming pattern, appetite	Walczak *et al.* (1983)
LAS	5.0	*Cirrhina migola*	Schooling pattern	Lal *et al.* (1984)
LAS	>0.015	Rainbow trout	Swimming endurance	Saboureau and Lesel (1977)
C_{10-13} LAS	0.2–0.4	Goldfish (*Carassius auratus*)	Swimming activity	Marchetti (1965)
C_{12}–C_{14} LAS	3.2–4.7	Flagfish (*Jordanella floridae*)	Feeding behavior	Foster *et al.* (1966)
ABS	10.0	Cod (*G. morrhua*)	Swimming activity	Swedmark *et al.* (1971)
LAS	0.5	*G. morrhua*	Swimming activity	
ABS	>1.0	Mussel (*Mytilus edulis*)	Byssal thread formation, Aductor muscle closing	
LAS	10.0			
Nonionic				
C_9 APE_9	2–4	Cod, mussel	Swimming activity, avoidance	Swedmark *et al.* (1976)
TAE EO(10)	0.5	*G. morrhua*	Swimming activity	Swedmark *et al.* (1971)
NP EO(10)[1]	>1.0	*G. morrhua*	Swimming activity	Swedmark *et al.* (1971)
NP EO(10)	5.0	*M. edulis*	Byssal thread formation, Adductor muscle closing	Swedmark *et al.* (1971)
NP EO(10)	2.0	Cockle (*Astarte montagui*)	Burrowing	Swedmark *et al.* (1971)
	5.0	Cockle (*Cardium edule*)	Burrowing	
	20.0	*Crangon crangon*	Locomotion	
	40.0	Decapod (*Leander adspersus*)	Locomotion	
		Hermit crab (*Eupagurus bernhardus*)		
		Shore crab (*Carcinus maenas*)		
	5.0	Barnacle (*Balanus balanoides*)	Cirral activity	
APE[2]	5–6	Rainbow trout	Swimming activity	A. D. Little Co. (1977)
C_9 APE_{10}	2.0	*G. morrhua*	Swimming activity, bysal activity	Swedmark *et al.* (1976)
NP[3]	0.002		Avoidance	Höglund (1976)
TAE-EO(10)	0.002	*G. morrhua*		
NP-EO(10)	0.002		Bysal activity, growth	Granmo *et al.* (1989)
4-NP	0.056	*M. edulis*		
Oleyl-cetyl alcohol-ethylene oxide condensate	<3.98	*Tilapia massambica*	Feeding	Chattopadhyay and Konar (1986b)

[1]Nonylphenol ethoxylate.
[2]Alkylphenol ethoxylate.
[3]Nonylphenol.

functional aspects of natural animal communities are unknown. Only a few studies have been reported describing the "long-term" effects of surfactants on natural zooplankton and invertebrate communities and these studies were conducted with LAS. Chattopadhyay and Konar (1985) reported that ostracods, rotifers and chironomids, in outdoor vats were adversely affected after 90 d exposure to 0.38–1.10 mg/l LAS based on the active ingredient. Zooplankton were reduced significantly at 0.51 and 1.10 mg/l. Huber *et al.* (1987) reported that 5 mg/l LAS adversely affected cyclopod egg production and developmental stages after 8 weeks exposure in model pond ecosystems. Egg production occurred at 3.5 mg/l LAS. Cladocera and phytoplankton were affected only after exposure to 10.0 mg/l. Ladle *et al.* (1989) found that sediment-bound LAS concentrations of 1–40 μg/g had no impact on the invertebrate diversity in a stream survey conducted above and below a municipal discharge. The effects of LAS in combination with a petroleum refinery effluent were investigated on phytoplankton, zooplankton and benthic organisms in outdoor ponds (Panigrahi and Konar, 1986). Combinations of 1 mg/l LAS with 0.4–13% effluent were toxic to zooplankton.

The range of reported chronic toxicity values for surfactants and aquatic animals is wide which can be attributed in part to the differences in experimental conditions. It is obvious that the toxicities of surfactants vary widely even within the same surfactant class (Fig. 2). Furthermore, toxicities of surfactants can vary with the chemical structure such as for LAS where the toxicity varies with the length of the alkyl chainlength (Kimerle and Swisher, 1977) and for the nonionic ethoxylated surfactants where toxicity varies with the length of the ethoxylate chainlength (Sivak *et al.*, 1982; Hall *et al.*, 1990). The range of effect and no effect concentrations based on the studies reviewed in this summary for fish was 0.05–28.0 mg/l (anionic surfactants), 0.05–50.0 mg/l (nonionic) and 0.05–0.46 mg/l (cationic). The range for invertebrates is 0.04–>10.0 mg/l (anionic), <0.1–20.0 mg/l (nonionic) and 0.009–1.27 mg/l (cationic). In previous surfactant toxicity reviews, chronic effect levels for aquatic animals were reported to range from 0.11 to 2.0 mg/l for alkyl ethoxylates (A. D. Little, Co., 1981) and to be as low as 0.1 mg/l for several major surfactants (Sivak *et al.*, 1982). Lewis and Suprenant (1983) reported that the acute toxicities of anionic, nonionic and cationic surfactants to aquatic invertebrates range, respectively, from 0.11 to 92.0, 0.21 to 500.0 and 0.08 to 2800.0 mg/l.

Sublethal effects data predominate for LAS and, with the exception of fish avoidance responses, the effect levels typically exceed 0.1 mg/l. The effect concentrations for nonionic surfactants, with a few exceptions, exceed 0.5 mg/l. Reported sublethal responses for cationic surfactants are too few to indicate a data trend.

Risk assessment

A relatively complete toxicity evaluation of a compound needs to include data for several test organisms (algae, invertebrate, fish) representing the trophic levels contained in the planktonic and benthic habitats of the environment (freshwater and saltwater) to which the compound is discharged. In addition, current measured environmental concentrations of the specific compound are needed since they would reflect recent usage rates, biodegradation and in-stream removal mechanisms and consequently provide the most realistic exposure scenario. Rarely are these data available for most chemical compounds and, with the exception of LAS and to a lesser extent for DTDMAC, this is true for most commercially important surfactants.

A brief description of the aquatic safety of representatives of the three major surfactant groups (LAS, alkyl ethoxylates, DTDMAC) follow based on the published data base and the generalized procedure of comparing laboratory-derived toxicity data with measured environmental concentrations.

Toxicity. The reported chronic effect concentrations have usually exceeded 0.1 mg/l for the various LAS blends, the alkyl ethoxylates (AE) and, in fewer cases, for DTDMAC (Fig. 2). These surfactants have been the more commonly tested and are commercially important representatives of the major surfactant groups used in detergent and softener products. The effect concentrations for the AE compounds, reviewed for this summary, were between 0.1 and 1.0 mg/l in all but one case and over 80% and 40% of the effect and no effect values for LAS exceeded 0.1 mg/l and 1.0 mg/l, respectively. The trend for DTDMAC is less clear due to the limited data base but results from standard toxicity tests with two commonly used test species have indicated an effect range of 0.1 to 1.0 mg/l when conducted in river water.

Exposure. Reported measured concentrations of specific surfactants in the environment have not been common until recently. The use of FAB mass spectrometry (Ventura *et al.*, 1989) and other analytical methodologies (Kikuchi *et al.*, 1989) will likely increase the availability of these data in the future. Currently, most reported environmental concentrations for surfactants are for LAS and the cationic DTDMAC in rivers receiving activated sludge treated municipal effluents (Table 5). Under these circumstances, and for the selected papers reviewed here, LAS concentrations in rivers have ranged from 0.0008 to 3.3 mg/l. Kimerle (1989) reported that although LAS concentrations of 0.001–10 mg/l have been reported for freshwater and marine waters, 85% of the values are between 0.01 and 0.1 mg/l, and 70% are between 0.01 and 0.05 mg/l. Concentrations of DTDMAC in various rivers have ranged from 0.001 to 0.092 mg/l. The reported values for LAS and DTDMAC, with few exceptions, are based on

Table 5. Measured environmental levels of surfactants as reported in the selected literature. Values, unless noted, represent range (mg/l)

Surfactant	Concentration (mg/l)	Location	Reference
Anionic			
ABS	BD[1]–0.54	Malaysia rivers and estuaries	Ludwig and Sekaran (1988)
		Ohio River	Woltering *et al.* (1987)
AES	0.008	Major U.S. rivers	A. D. Little Co. (1977)
LAS	0.01–3.3	Unamed U.S. river	Osburn (1986)
LAS	0.01–0.27	U.K. rivers	Gilbert and Kleiser (1986)
LAS	0.04 (0.008–0.17)	Tokyo Bay	Kikuchi *et al.* (1986)
LAS	0.0008–0.030	German rivers	Topping and Waters (1982)
LAS	0.28 (0.08–0.61)	Town River, Mass.	Lewis and Wee (1983)
LAS	0.04–0.59	German rivers	Matthijs and de Henau (1987)
LAS	0.04 (0.01–0.09)	Seawater	Martinez *et al.* (1989)
LAS	0–0.26	Ebro River	
	0–0.34	Eight U.S. rivers	Hennes and Rapaport (1989)
LAS	0.01–0.04	Eleven European rivers	
	0.01–0.09		
Nonionic			
Alcohol ethoxylates	0.01–1.0	Several European rivers	A.D. Little Co. (1977)
Cationic			
DTDMAC	0.004–0.092	Rhine River Basin	Kappeler (1982)
DTDMAC	0.013–0.037	U.S. river	Wee (1984)
DTDMAC	0.033 (0.001–0.092)	Rapid Creek, S.D.	Lewis and Wee (1983)
DTDMAC	0.017 (0.009–0.028)	Blackstone River, Mass.	Lewis and Wee (1983)
DTDMAC	0.024 (0.012–0.040)	Otter River, Mass.	Lewis and Wee (1983)
DTDMAC	<0.002	Millers River, Mass.	Lewis and Wee (1983)
DSDMAC	0.008 (0.002–0.016)	German river	Topping and Waters (1982)
	0.014 (0.009–0.02)	U.K. river	
C_{12-18} MAQ[2]	BD–0.012	31 European and U.S. rivers	Woltering *et al.* (1987)

[1]BD = below detection.
[2]MAQ = monoalkyl quaternary ammonium salt.

chemical-specific methodologies. In contrast, routine analytical methods for specific nonionic surfactants have not been reported. A. D. Little Co. (1977) summarized the reported concentrations of nonionic surfactants in several European rivers. The range of concentrations was 0.01–1.0 mg/l which represented total "nonionic substance". It was reported in the A. D. Little Co. review that no reports of nonionic surfactant levels in the U.S.A. were found.

Toxicity–exposure comparison. In most cases, a safety margin is indicated based on the comparison of the more commonly observed toxicity values (>0.1 mg/l) and exposure data for LAS. The significance of the infrequent overlap in the exposure and effects data for LAS is unknown but should not be a major concern due to the site-specific and sometimes non-specific nature of the measured environmental concentrations and to most field-derived toxicity results that show LAS to be relatively non-toxic to natural animal communities (Chattopadhyay and Konar, 1985; Huber *et al.*, 1987). It appears that DTDMAC is not an obvious environmental hazard based on the available data. However, this conclusion is less technically supported than is that for LAS. Effects on saltwater environments and on natural freshwater animal communities are key unknowns that need to be determined before the environmental safety of this and other similar cationic compounds can be confidently assumed. Measured concentrations of specific AE compounds in the environment are needed to confirm the predicted safety of these nonionic surfactants.

The environmental impacts of LAS and DTDMAC have been discussed by the international scientific community (German Chemical Society, 1982; Ruchay, 1982). In addition, LAS has been reported to be environmentally safe in a variety of reports (Gledhill, 1974; Sivak *et al.*, 1982; A. D. Little Co., 1981; Gilbert and Pettigrew, 1984; DeHenau *et al.*, 1986; Huber, 1989; Kimerle, 1989; Martinez *et al.*, 1989). Of these papers, Kimerle's is the most thorough evaluation of the subject. The environmental data base for LAS is the most extensive of any surfactant (Kikuchi *et al.*, 1986; Huber, 1989) and a review of the data summarized in this report for planktonic and benthic animal life and for aquatic vegetation (Lewis, 1991) supports the aquatic safety of this compound more so than for any other surfactant. A detailed discussion of the environmental safety of LAS can be found in *Tensides Surfactants Detergents* (Vol. 26, No.2).

Risk assessments for the softener active DTDMAC based on laboratory toxicity data (Lewis and Wee, 1983) and on laboratory and field-derived data for algae and phytoplankton (Lewis and Hamm, 1986; Lewis, 1991) predict the likelihood of safety in freshwater. It can be stated with more certainty that DTDMAC adsorbed to sediment is probably non-toxic to freshwater benthic life. Lee (1986) and Pittinger *et al.* (1989), have reported the non-toxic nature of sediment bound cationic compounds to midge. In contrast, Lahl and Zeschmer (1986) recommended that cationic surfactants as well as a variety of other detergent ingredients

not be included in "environmentally safe" detergent products. Several Western European countries are debating DTDMAC "bans". Published reports for other cationic surfactants are either uncertain on safety primarily due to the lack of field and exposure data (Cooper, 1988) or predict safety based on the available data (Woltering *et al.*, 1987). Detailed safety assessments for most nonionic surfactants have not been reported, but the safety of alkyl ethoxylates has been concluded (Kravetz *et al.*, 1986; Shell Chemical Co.). In contrast, nonylphenol based ethoxylates, a major class of industrial surfactants, and their biodegeneration by-products are toxic and recalcitrant (Brunner *et al.*, 1988) and their environmental safety is highly questionable.

Overall, the data summary and brief risk assessment presented here shows that a comprehensive effect and exposure data base exists for aquatic animal life and LAS but that comparable information for other surfactants is either unknown or unreported. Therefore, safety evaluations for most surfactants in freshwater and more so in saltwater environments should be considered limited and preliminary in nature since they are based largely on toxicity and exposure predictions in need of validation. With this in mind, continued generation of environmental effects and exposure data for LAS should be of low priority when compared to the obvious need for these data for other widely used surfactants.

Sublethal effects

The utility of behavioral and physiological effects data in estimating an environmental impact is unknown in most cases (Rand, 1985) and has been rated below the value of chronic test results (Macek *et al.*, 1978). This lack of predictive value is attributable to a variety of factors including lack of established methodologies, incomplete understanding of the physiology of aquatic organisms and the current inability to relate biophysiological and behavioral changes to the health and survival of the organism. It is obvious that avoidance responses are a sensitive effect parameter when compared to the other effect parameters reported for surfactants. However, their predictive capability for estimating chronic effects is limited due to a lack of field validation and also to the lack of a statistical correlation of avoidance concentrations and chronically toxic levels (Giattina and Garton, 1983; Smith and Bailey, 1989). Furthermore, in several reports the subiethal effects were reversible (Maciorowski *et al.*, 1977; Olsen and Hoglund, 1985). The utility of sublethal effects data in the safety assessment process will increase in the future as their environmental relevance is investigated. When this occurs, priority should be assigned to determining these effects for the cationic surfactants.

OVERVIEW AND RECOMMENDATIONS

Historically, the effects of the anionic ABS (alkylbenzene sulfonate) and LAS have been the primary focus of scientific investigations concerning surfactant environmental safety. The importance of the nonionic and cationic surfactants to the detergent industry has increased during the past 15 years. Consequently, their use in toxicity tests has increased, but the cationic surfactants still do not receive the attention they deserve. The reported toxicity data base for surfactants is dominated by data for freshwater species which reflects, in part, the availability of standard test methods and suitable culture techniques. This contrasts the lack of standard methods and culture techniques for marine species which have been available only until recently. Consequently, the toxicities of surfactants on saltwater life have been and continue to be largely estimated from effects derived on freshwater life. Since this practice is not technically sound, in most cases, toxicity data for saltwater species are needed. Based on the data in this review and that reported earlier for algae (Lewis, 1991) the chronic toxicities of surfactants for freshwater aquatic plants are better understood, particularly on natural communities, than are those for animal life. With the exception of cationic surfactants, algae are not sensitive to surfactants. It even appears that the toxicitity of the cationic surfactants observed in the laboratory for single algal species is less for natural phytoplankton communities although additional supporting data for periphyton communities are needed. Overall, future investigations concerning the environmental safety of surfactants should center on understanding their chronic toxicities to animal life.

Animal test species have exhibited a moderate degree of sensitivity to several major anionic and nonionic surfactants but at concentrations typically exceeding, where available, measured environmental concentrations. The few available effect and no effect levels for cationic surfactants appear to occur at lower concentrations than those for many anionic and nonionic surfactants but a definitive trend cannot be identified at this time due to the limited nature of the data base.

Commonly used surfactants for which a limited chronic toxicity data base exists include the alkyl sulfates (anionic), alkyl ethoxylate sulfates (anionic) and several of the monoalkyl and dialkyl quaternary ammonium salts (cationic). Of these compounds it would be expected, based on the available information for aquatic animal and plant life, that the toxicities of the anionic compounds would likely parallel those for LAS and be relatively nontoxic. However, the use of alkyl sulfates and alkyl ethoxylate sulfate in commercial products is substantial and increasing, and selected chronic toxicity determinations with these surfactants would determine if their toxicities are comparable to LAS. The key

scientific need for nonionic surfactants is to determine the environmental concentrations in freshwater and saltwater environments of the major alkyl ethoxylates and compare these to the available toxicity data base. This need for a realistic exposure analysis includes most other surfactants as well. The use of the nonionic alkylphenol exthoxylates, particularly several of the nonylphenol ethoxylates is decreasing due in part, to their environmental toxicity and legislative action in Western Europe to ban their use (Richtler and Knaut, 1988). Therefore, they should be of a low priority from a research perspective. The toxicity data base for cationic surfactants needs to be expanded. The chronic toxicity tests needed for these surfactants are chemical-specific but would include baseline toxicity studies with saltwater and freshwater laboratory fish and invertebrates, tests investigating physiological and behavioral effects, and of greater priority, those determining effect levels for natural freshwater and saltwater animal assemblages.

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ATTACHMENT #13

Women's Health Today: Perspectives on Current Research and Clinical Practice.
D.R. Popkin and L.J. Peddle, eds. Parthenon Publ. Group, New York, pp. 201-207. (1994).

Section 9: Environmental factors affecting fertility

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Endocrine-disrupting environmental contaminants and reproduction: lessons from the study of wildlife

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Introduction

Human agricultural and industrial activity has released an enormous array of chemicals into the environment. Many of these persistent xenobiotic (i.e. man-made) contaminants exhibit low vapor pressures ($< 10^{-5}$ atm), and are lipophilic in nature; thus, they have high solubilities in non-polar liquids and low solubilities in water (< 1mg/l)[1]. Low vapor pressures augment contaminant distribution by atmospheric transport, so that, at present, global areas perceived as pristine (e.g. polar regions) have measurable contamination levels with such persistent compounds as DDT (and its metabolite DDE), polychlorinated biphenyls (PCBs) and dioxin (TCDD). The lipophilic nature of these compounds allows them to be readily assimilated in the lipid stores of plants and animals; thus, these compounds rapidly enter the food web of all ecosystems. The assimilation and persistence of xenobiotic compounds at various levels of the food web enables the bioaccumulation and biomagnification of these factors. Throughout its lifetime, an organism feeding in a food web will consume or absorb compounds that are retained in its tissues and are not further degraded, i.e. these compounds are bioaccumulated. All organisms bioaccumulate environmental compounds to some degree but those species feeding at higher levels of the food web will also exhibit biomagnification because they eat animals that have also bioaccumulated compounds (Figure 1). These two factors can produce a condition where top-level predators carry body burdens of contaminants at levels many million times greater than the environmental background level. For example, the PCB concentration in herring gull eggs in some regions of the Great Lakes (USA) is 25 million times greater than the concentration measured in the water[2]. Likewise, humans living near the Great Lakes have PCB levels significantly greater than backgound (Figure 1), with those individuals who regularly eat fish caught in the lakes themselves exhibiting the greatest biomagnification.

It has been reported that xenobiotic compounds – such as DDT, DDE, some PCBs, dieldrin, and dioxin – are potent disrupters of reproductive activity[3]. This fact became common public knowledge in the early 1960s with the publication of Rachel Carson's *Silent Spring*[4]. However, the focus for most studies linking contaminants and wildlife or human health has centered on the occurrence of cancer or mortality as end-points. Recent studies, summarized in Colborn and Clement[5], suggest that a new set of end-points must be implemented, as evidence is rapidly accumulating that xenobiotic compounds are capable of acting as agonistic and antagonistic agents of the endocrine system (Figure 2). Xenobiotic contaminants can disrupt or modify embryonic development and consquently modify the functioning of various adult organ systems (e.g. reproductive, immune and neurological)[5]. The fact that xenobiotic contaminants have multigenerational effects has profound implications for public health, the maintenance of wildlife populations and future economic development.

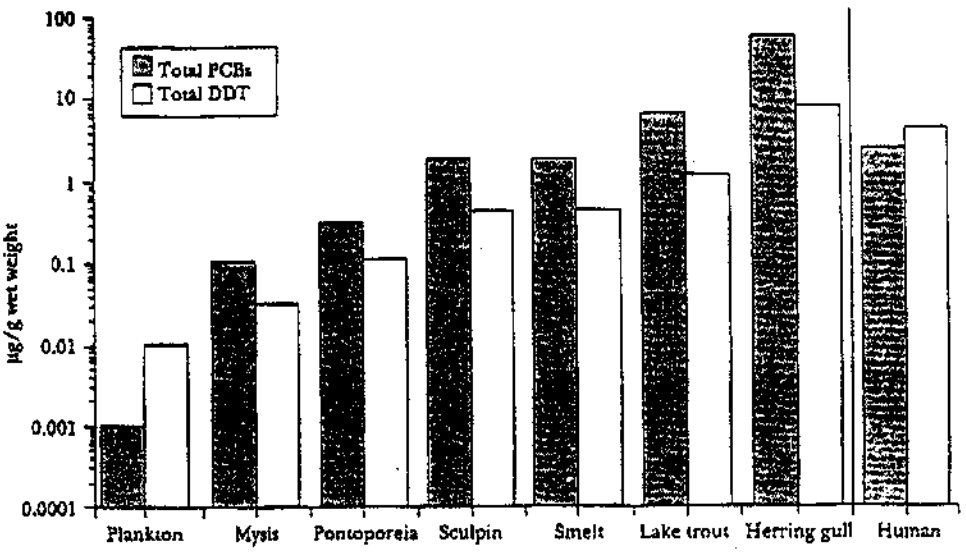

Figure 1 Examples of biomagnification in the food web for two persistent contaminants, DDT and PCBs. Wildlife data are taken from Colborn *et al.*[2] for samples from Lake Ontario, Great Lakes, USA. Human data (representing mean wet weight concentrations for adipose tissue) were obtained from the study of Williams *et al.*[22] for individuals from Ontario, Canada. Note that y axis is a log scale

The problem

In 1979, the National Institutes of Environmental Health Science (USA) organized a symposium entitled 'Estrogens in the Environment' to address a growing concern that many compounds released into the environment, such as diethylstilbestrol (DES), used in the animal science industry, and the pesticide DDT, were known to have estrogenic activity[6]. These same concerns were addressed over a decade later in 1992 at a conference organized by the Worldwide Fund for Nature[3], where scientists interested in both human and wildlife health issues addressed the premise that xenobiotic compounds were acting not only as estrogens but were disrupting the endocrine system and, thus, modifying developing embryos and so permanently altering the reproductive, immunological and neurological capabilities of future populations. The scientists at that meeting concluded: 'We are certain of the following: a large number of man-made chemicals that have been released into the environment . . . have the potential to disrupt the endocrine systems of animals, including humans'[3].

What are the data supporting this statement? Should wildlife health problems be used as indicators of current or future human health concerns? These questions cannot be addressed completely in this paper, but data are provided that suggest that not only are wildlife excellent models for attempting to understand some human health concerns, but they may also be used as predictive models of additional health problems that are not traditionally examined in studies of human health and environmental contamination.

Specific effects of contaminants

The detrimental effects of environmental contamination on reproductive and endocrine function have been documented repeatedly in the scientific literature[3]. Although causal agents

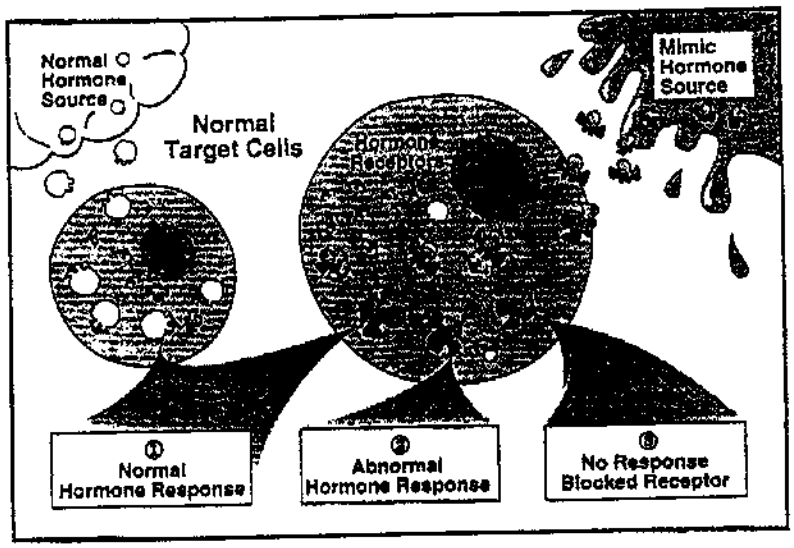

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Figure 2 Environmental contaminants have been shown to mimic naturally occurring hormones. Hormones stimulate or inhibit various functions in cells by binding with a receptor molecule. The hormone–receptor complex then interacts with either the cell DNA or with second-messenger systems to produce specific actions (e.g. protein synthesis, cAMP turnover). Contaminants that mimic hormones can duplicate the normal hormonal process (shown in 1) but they may also interact with the receptor in a manner which stimulates an aberrant function (2) or may block the receptor producing no function (3)

and effects are known in some cases, the underlying mechanism(s) are still poorly understood and require extensive research[7–9]. The reproductive disorders reported to date in wildlife involve such factors as reduced fertility, reduced hatchability, reduced viability of offspring, poor growth, wasting and lower rates of activity in neonates, impaired hormone activity, and modified adult sexual behavior (Table 1). Abnormalities of these types can be caused by disruption of normal endocrine function, either prior to or after the hormone interacts with specific cellular receptors (Figure 2). For example, a number of the effects described above are correlated with decreased circulating concentrations of sex steroids or thyroid hormones (Table 1). Causal linkages between xenobiotic agents and observed effects in wildlife and human populations have been difficult to establish due to varying analytical methodologies; use of differing and various endpoints of effect; species differences; age differences; sex differences; and potential interaction between chemicals.

Extensive laboratory studies using traditional laboratory methodologies of toxicology have shown causal relationships between specific impairments of reproductive activity and xenobiotic agents (for examples, see refs. 10–12). However, the majority of wildlife studies, as with human studies, have relied on correlative relationships between elevated tissue concentrations of various contaminants and specific reproductive abnormalities (for examples, see the reported studies on white croakers[13], double-crested cormorants[14] and alligators[15]). Although correlation does not imply causation, recent wildlife studies have used the systematic methodologies of epidemiology – 'ecoepidemiology' – to evaluate the relationships between a suspected xenobiotic agent and disease-states in wildlife and human subpopulations (see the study by Fox[16] for a description of these techniques, and Bishop and colleagues[17] for an example of their application). Studies using this approach have now provided strong evidence that various environmental contaminants dis-

Table 1 Discrete effects of environmental contamination on reproductive characteristics of selected wildlife species (partial, representative listing only)

Wildlife	*Effect*	*Correlation*	*Reference*
Fish			
Salmon	premature sexual maturity, loss of sexual dimorphism, ↓17β-estradiol, ↓dihydroxyprogesterone, ↓fertility, ↑embryo mortality	PCBs, DDT, dioxins, furans, heavy metals	23
Burbot	no sexual maturity, ↑sterility	PCBs, DDT, dioxins, furans	24
White croaker	↓fecundity and fertility, ↑embryonic mortality, ↑ovarian follicular atresia	DDT	13
Reptiles			
Snapping turtle	↑embryonic mortality and deformities	PCBs, dioxin, furans	17
American alligator	↓testosterone (male), ↑17β-estradiol (male and female), polyovular follicles and polynuclear oocytes	DDE	15
Birds			
Bald eagle	↓fertility, ↑embryonic mortality and deformities	PCBs, DDE, dieldrin	25
Japanese quail	↓17β-estradiol prior to sexual maturation; delayed oviposition, ↓laying capacity	PCBs	26
Mammals			
Dall's porpoises	↓plasma testosterone	p,p'-DDE	27
Beluga	↓follicular activity, mammary carcinoma	DDT, Mirex, PCBs	28

rupt the embryonic development of the reproductive system of numerous wildlife species, permanently altering the reproductive capabilities of these individuals (Tables 1 and 2).

It is apparent that one mechanism by which some environmental contaminants influence the reproductive system is via the estrogen receptors[6–8]. Laboratory studies using DES treatment on the neonatal mouse model and medical observations of effects of DES exposure on humans have shown that early exposure to estrogenic compounds has both subtle and catastrophic (carcinoma) effects on the reproductive system (Table 2). The subtle effects in female mice include changes in, first, the structure of the ovarian follicle and oocyte (polyovular follicles and polynuclear oocytes); second, in the number of receptors for estrogens (E_r), progestins (P_r) and epidermal growth factor (EGF_r) in the vagina; and, third, in the E_r and P_r in the uterus and mammary gland[7,18]. Similar modifications in receptor number are seen in the prostate and seminal vesicle of males. Additional studies demonstrated numerous changes in the protein secretion patterns of the reproductive tract of mice following neonatal exposure to DES, that were indicative of changes in gene expression[19,20]. Interestingly, recent evidence suggests that an interaction between xenobiotic chemicals and the estrogen receptor may not be the only mechanism by which disruptions of the development of the reproductive system occur. Evidence now exists that the metabolites of at least one contaminant (the fungicide vinclozolin) actively bind to the androgen receptor and demasculinize developing male rats via their antiandrogenic activity[9]. Thus, many of the developmental abnormalities, both anatomical and physiological, of the reproductive tract, attributed to the 'estrogenic activity' of various contaminants, may in fact be due to the antiandrogenic activities of the same compounds (L. Earl Gray, personal communication). These studies suggest that we need further research on the developmental effects of environmental contaminants because we are only beginning to understand the cellular and genetic consequences of such exposure.

Table 2 Abnormalities of the reproductive system in women, laboratory animals and wildlife associated with exposure to endocrine-mimicking or -disrupting contaminants during embryonic development. DES = diethylstilbestrol; DDT = 2,2-bis (*p*-chloro phenyl) 1,1,1,-trichloro-ethane; PAHs = polycyclic aromatic hydrocarbons; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. This is a partial and representative listing only; data were compiled from selected references. See particularly ref. 2, 3, 5, 7, 21 and 23

Effect	*Human*		*Laboratory animal*		*Wildlife*[†]	
Reproductive tract malformations	yes	DES	mouse rat hamster Western gulls	DES TCDD TCDD DDT	Western gulls herring gulls Baltic ringed seals	DDT DDT, DDE and PCBs PCBs
Polyovular ovarian follicles	yes	DES	mouse	DES	alligator	DDE and Dicofol
Polynuclear oocytes	?		mouse	DES	alligator	DDE and Dicofol
Paraovarian cysts Polycystic ovary	yes yes	DES DDT	mouse	DES	?	
Carcinoma	vagina mammary*	DES DDT	mouse (vagina, mammary)	DES	Beluga whale (mammary)*	DDT, PCBs, Mirex
Abnormal gonadotropin synthesis	?		rats quail	TCDD Parathion	?	
Abnormal steroid synthesis	yes	PCBs	rats primates	TCDD TCDD	quail salmon alligator	PCBs PCBs, DDT, TCDD and furans DDE and Dicofol
Irregular menstrual cycle Persistent vaginal estrus Irregular vaginal estrus	yes	PCBs and PCDFs	rats hamster rats and mice	DDT TCDD Chlordecone	?	
Oocyte destruction/atresia	?		mice chicken	PAHs DDT	white croaker	DDT
Endometriosis	?		Rhesus monkey	TCDD	?	
Sub- or infertility	yes	DES	mice hamster	DES TCDD	Beluga whale white croaker	DDT, PCBs and Mirex DDT

*Correlative relationship only. †All relationships indicated are correlations between reproductive abnormalities and contaminants present in tissues or eggs at the greatest concentration

Within the realm of endocrine-disrupting environmental contaminants, how predictive of human reproductive health problems are laboratory and wildlife models? Although no definitive answer to this question is currently possible, it is clear that many of the reproductive health defects observed in wildlife living in contaminated areas, or exposed to contaminants under controlled laboratory conditions, mimic closely similar conditions found in humans and laboratory animals (see Table 2). The similarity in reproductive abnormalities observed in women and mice exposed to DES at similar developmental stages has supported the view

that laboratory models can be used to predict detrimental human health conditions[21]. Laboratory studies, however, typically involve treating the experimental subjects with one compound and noting its effect(s) over a relatively short period of the animal's lifetime. Humans and wildlife are not exposed to single compounds at a single time in their lives but are constantly exposed to a wide range of xenobiotic agents. The majority of the human populations currently under study for contaminant-induced health problems have not been examined for the subtle reproductive and endocrine defects reported in wildlife. Thus, wildlife can serve as important sentinels of ecosystem health and human risk. An ecoepidemiological approach – examining wildlife reproductive abnormalities combined with traditional toxicological studies – to identify causation may provide the best model for predicting human reproductive health concerns.

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ENVIRONMENTAL ESTROGENS

New Yeast Study Finds Strength in Numbers

The notion that modern industrial society is producing hormonelike pollutants that can interfere with human reproduction has become a hot topic in the media and within Congress in recent months. A widely promoted book, *Our Stolen Future* (see review on p. 1444), with a preface by Vice President Al Gore, put the theory high on the public agenda—and drew a strong response from some researchers who pointed out that the pollutants don't have nearly the clout of natural estrogens in the body and, thus, may have no significant impact on humans. Now, a paper in this issue (p. 1489; also see Perspective on p. 1451) is likely to add fresh fuel to the debate. A team of researchers from Tulane University in New Orleans, using a novel screening system based on genetically engineered yeast cells, reports that a mixture of two weakly estrogenic chemicals can be far more potent than the individual compounds.

The findings are causing scientists to take a fresh look at the controversy. "It's a very striking result," says Wade Welshons, an endocrinologist at the University of Missouri. "It doesn't forge a direct connection between developmental estrogen problems and these chemicals, but it's a very important red flag." Others caution, however, that more work must be done to pin down whether the mechanism found in yeast cells has any relevance to humans. "These are very interesting observations, but they raise more questions than they answer," says Jack Gorski, a biochemical endocrinologist at the University of Wisconsin.

The Tulane research addresses one of the hottest controversies in toxicology: Do estrogenlike compounds in the environment—for example, pesticides, the plastics ingredient bisphenol-A, and some polychlorinated biphenyls (PCBs)—contribute to such ills as breast cancer, a possible drop in human sperm counts, and a rise in testicular cancer (*Science*, 15 July 1994, p. 308)? Some researchers have linked spills of such chemicals with reproductive abnormalities in wildlife, but the debate centers on whether the low levels present in the environment are sufficient to harm humans.

Strong synergy. Tulane's Collins, Klotz, McLachlan, and Arnold test combinations.

Two years ago, Tulane environmental endocrinologist John McLachlan, then scientific director of the National Institute of Environmental Health Sciences, and collaborators came upon a possible clue. The group was able to make male turtle embryos develop into females by painting the eggs with estradiol—the body's main estrogen—or certain estrogenic PCBs. At moderate doses they achieved this effect only when they combined two PCB compounds; the same PCBs applied individually were ineffective. So 6 months ago, when McLachlan teamed with molecular endocrinologist Steve Arnold and University of Florida reproductive physiologist Louis Guillette to set up a yeast system to screen for environmental estrogens, they decided to test various mixtures of the compounds.

The system consists of yeast cells engineered to contain genes that code for the human estrogen receptor and a "reporter" protein that the cell makes when an estrogenlike compound binds to the receptor. The culture turns blue when a chemical binds to the receptor, and the intensity of the color reflects how strongly the receptor is activated.

Tests on four pesticides believed to be only very weakly estrogenic—the pesticides dieldrin, endosulfan, toxaphene, and chlordane—yielded little or no response, as expected. (All but endosulfan have been banned in the United States, but they persist in the environment, sometimes in combination.) When the chemicals were paired, however, the activity shot up by a factor of 160 to 1600. "It was really quite astounding," McLachlan says. The group also found a fivefold synergistic effect in the yeast cells with a PCB mixture that had reversed the sex of the turtle eggs. And they showed that their results were not specific to the yeast system by getting comparable effects with PCBs in human endometrial cells.

Although the various combinations of pesticides were only 1/500 to 1/15 as potent as estradiol itself, McLachlan says his group worked with "levels [of environmental estrogens] actually achieved in some systems," such as the turtle eggs and PCBs in the serum of a group of women with breast cancer. The results, he says, "at least provide a mechanism where low levels of weak-acting environmental estrogens could have a greater-than-expected effect."

Other researchers emphasize that the results must be verified in various animal species to establish whether they are relevant to wildlife or people. The yeast-cell system "is a good controlled experimental system. But these are the first observations from the system," says toxicologist Michael Gallo of the Robert Wood Johnson Medical School in New Jersey. "[Now researchers] have to move into different phyla and ratchet down on the molecular explanation." McLachlan's team is now studying the estrogen receptor's binding pockets in search of a molecular mechanism.

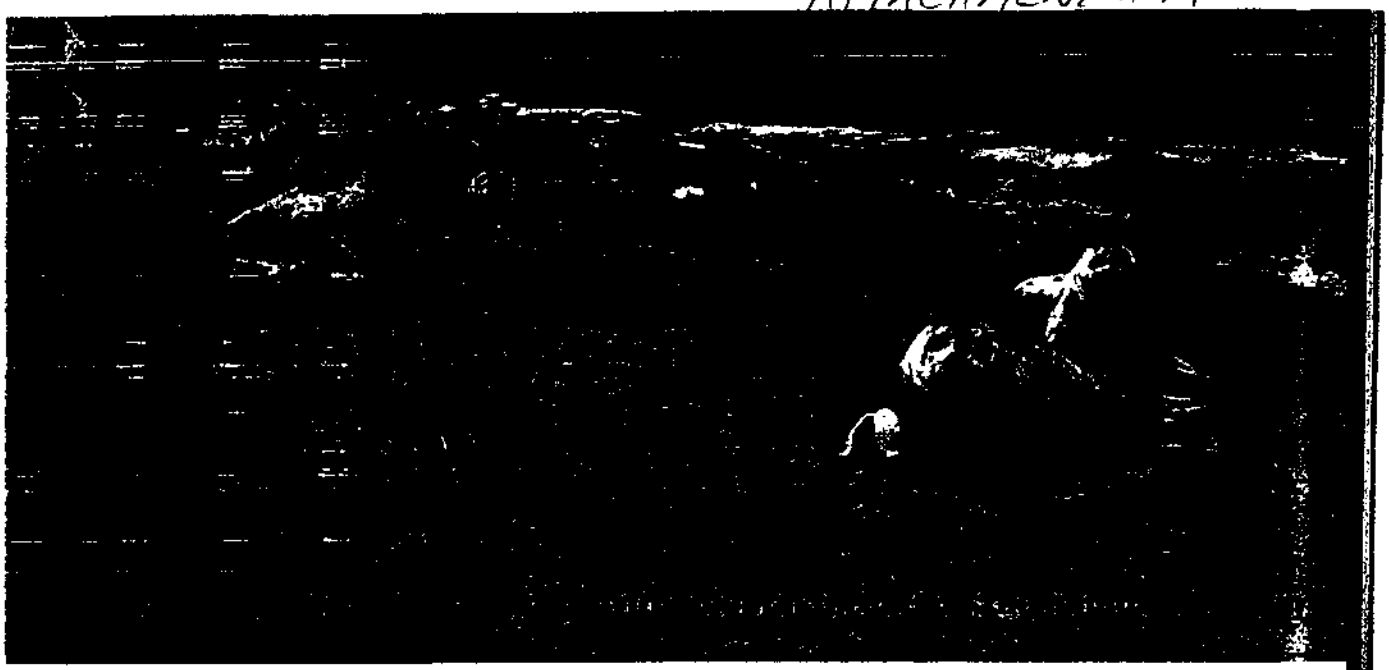
Toxicologist Stephen Safe of Texas A&M University, a vocal skeptic of the notion that environmental estrogens are linked to human health effects, agrees that the findings "are really interesting and may have environmental significance." But he says the data do nothing to undermine a major criticism of the hypothesis: that many synthetic and natural environmental estrogens, including some in plants, are actually "antiestrogenic"—they block or reduce the activity of estrogen receptors—and could cancel out even powerful synergistic estrogenic effects. "We have to look at the opposite side of the coin," Safe says. McLachlan acknowledges this possibility and says his group has begun testing antiestrogenic chemicals and estrogenic/antiestrogenic combinations.

For now, the findings will stimulate more studies of chemical cocktails—an area largely overlooked in recent research on endocrine disrupters, which has focused on individual compounds. And if the results do hold up in various animal species, scientists may need to revise their current assumption that the effects are additive. "The safety margin may be a lot smaller than has been anticipated," says toxicologist John Gierthy of the New York State Department of Health. It could also "make testing extremely complex," he adds.

Indeed, the results may need to be taken into account by an Environmental Protection Agency (EPA) advisory panel now being formed to come up with in vitro test strategies to screen for environmental estrogens that pose the greatest potential threat, says Lynn Goldman, head of the EPA Office of Prevention, Pesticides, and Toxic Substances. Legislation pending in Congress would require EPA to begin screening such chemicals within 2 years. The Tulane findings could have "enormous policy implications" for EPA, says Goldman. "Obviously," she says, "these systems are more complex than we had imagined."

–Jocelyn Kaiser

ATTACHMENT #14



In the North and Baltic Seas over several months in early 1988, some 25,000 harbor seals – about 60 to 70 percent of those residing there – abruptly perished. Subsequent investigation would trace this, the largest seal die-off in history, to a novel germ.

Dutch virologist Albert D.M.E. Osterhaus of Erasmus University in Rotterdam helped identify the source of the lethal epidemic: a distemper virus – one that he describes as "quite similar" to a microbe that can cause serious respiratory, gastrointestinal, and central nervous system problems in dogs.

But having solved one mystery – what killed the seals – he had found another: What had rendered these mammals so vulnerable? Now, he has data that illustrate the unintended injury that can result from industrial activities.

While the seals were dying, Osterhaus recalls, "There was a lot of controversy about pollution being the major cause." And even after his group successfully indicted the viral perpetrator, he says, "We held open the possibility that this particular virus – or its effects – might have been aggravated by preexposure of the animals to certain pollutants."

Had this epidemic been an isolated event, it might have entered the annals of biology as just another curiosity. But in fact, notes Susan D. Shaw, executive director of the New York City-based Marine Environmental Research Institute (MERI), this die-off was but the most tragic manifestation of a worrisome trend.

Over a 5-year period beginning in 1986, green sea turtles began appearing with massive tumors known as fibropapillomas. MERI reports that up to half the world's population of this endangered species may now bear such tumors, which have been linked to infection with a herpeslike virus.

During 1987 and 1988, some 750 bottlenosed dolphins died along beaches from New Jersey to Florida – many having succumbed to pneumonia and severe, exfoliating skin lesions that resembled acid burns. Where tested, the blubber of these animals often contained very high concentrations of chemicals called polychlorinated biphenyls (PCBs).

In 1987, seals in Siberia's Lake Baikal died in large numbers from a distemper virus. A year later, white-sided dolphins experienced a mysterious and lethal epidemic in the waters off Lubec, Maine.

Two years later, the remains of 274 bottlenosed dolphins were recovered along the U.S. shores of the Gulf of Mexico – many of them covered in a mysterious fungal growth. Between 1990 and 1992, more than 1,000 Mediterranean striped dolphins succumbed to an infection resembling the one that devastated the harbor seals in 1988. High concentrations of PCBs turned up in many of the dolphin carcasses examined – both in the Gulf and the Mediterranean.

Finally, in 1991, 39 harbor seals were found stranded or dead off the New York coast – 62 percent more than a year earlier. Investigators found not only high concentrations of PCBs in the few seals tested, but also low counts of the white blood cells that fight off infection, MERI reports.

Though apparently unrelated, all these episodes involved populations that carried high burdens of toxic organochlorines – such as PCBs and dioxins – in their bodies or that lived in waters polluted with high quantities of toxic industrial chemicals. Because studies have demonstrated that PCBs and certain other organochlorines can suppress the infection-fighting immune system of laboratory animals, Shaw says that a circumstantial case began to build linking pollution to the viral epidemics devastating animals at the top of the marine food chain.

Indeed, such concerns prompted Osterhaus and his colleagues at the National Institute of Public Health and Environmental Protection in Bilthoven, the Netherlands, to launch a unique study involving harbor seals (*Phoca vitulina*). Now, three years later, they're able to report data strongly supporting their initial suspicions – that pollution may affect vulnerability to disease.

The Dutch team captured pups living along the northeast coast of Scotland. For a year, the researchers fed the animals a normal diet consisting of herring. Over the subsequent 2-year feeding trial, the 14 females and 8 males continued to eat herring. Half received fish from the relatively clean North Atlantic; the rest dined on fare harvested from the industrially polluted Baltic.

Chemical analyses showed that the Baltic fish carried 10 times as much toxic organochlorine contamination as did the North Atlantic herring. Osterhaus points out, however, that all the fish came from catches that had been destined for human consumption.

Every 6 to 9 weeks, the researchers took blood samples and made various measurements of the animals' immune functioning. Though both groups exhibited identical baseline values for the different factors, those fed Baltic herring quickly distinguished themselves once the study began.

For instance, the concentrations of

vitamin A in their blood dropped by 20 to 40 percent almost immediately and remained low throughout the 2 years. While the importance of this change is open to interpretation, Shaw notes that in other animal models, "vitamin A has been linked to disease resistance, with lower [blood] concentrations corresponding to increased vulnerability."

As vitamin A was falling, the concentrations of granulocytes in the blood of the Baltic-fed seals climbed and remained consistently elevated – at about 10 to 15 percent above those in the other group. Because this subpopulation of white blood cells is important in combating pathogenic bacteria, Osterhaus now speculates that seals eating the more polluted herring may have suffered from higher levels of chronic infections.

Even more impressive was an almost immediate plummeting in the activity of another population of white blood cells, known as natural killers (NK). These can attack foreign bodies without first having to recognize specific antigens – molecular "flags" that help trigger an immune response. NK activity in seals fed Baltic herring remained 20 to 50 percent below normal throughout the study, reflecting an apparent drop in the responsiveness of this important facet of the animals' immune system.

Another crucial component of the immune system also appeared somewhat depressed in seals eating the more polluted fish. B cells, which produce antibodies, and T cells, which orchestrate or directly participate in an animal's immune defense, will ordinarily proliferate when challenged with various types of antigens.

During their study, the scientists report in the March AMBIO, the proliferative response of the T cells to a set of standard antigens dropped 25 to 60 percent in the seals fed Baltic herring, compared to those fed fish from the Atlantic. Additional, unpublished data suggest that the antibody responses of B cells also were impaired.

"I was surprised to see significant immune changes in animals that were fed on a normal diet using fish fit for human consumption," Osterhaus says. Extrapolating these findings back to the 1988 Baltic and North Sea distemper die-off, he adds, "I think we can now say that it's not unlikely that the seals' natural immune response to infection may have been impaired."

Molecular immunologist David A. Ferrick of the University of California School of Veterinary Medicine in Davis describes the feeding trial as a landmark study. "This is the first time anybody's done a good, controlled study looking at the direct effect of contaminant exposures on immune function or ability to resist disease," says Ferrick,

Peter Ralston

Maine harbor seal basks on ledge of rock in outer Penobscot Bay. Such blubbery marine mammals accumulate fat-seeking pollutants, some of which may affect an animal's vulnerability to disease.

codirector of the International Program for Marine Mammal Health at the Marine Mammal Center in Sausalito, Calif.

Indeed, he notes, owing to the heavy regulatory protection afforded marine mammals by many nations, such a feeding trial – involving food known to be heavily contaminated – probably could not win approval in most countries, the United States included.

However, while describing the study's findings as interesting and important, Ferrick cautions against overinterpreting them. "The immunological differences that [the Dutch team] measured are not what you would call astounding" or even substantial enough to guarantee that they would compromise an animal's ability to fight infection.

But Ferrick also points out that the study animals were well maintained in captivity, with adequate stores of food. The immune responses observed in the seals fed Baltic herring may simply reflect the steady, chronic – and *subtoxic* – ingestion of potentially dangerous pollutants.

Many of the toxic chemicals detected in these fish accumulate in fat. "And their large blubber stores mean that marine mammals can accumulate far greater quantities of these contaminants than can most species," Ferrick notes.

In the wild, marine mammals periodically draw down that fat. It provides much of the energy that a mother delivers to her suckling young, it can also ensure an individual's survival during the periods of famine that inevitably seem to follow periods of abundance.

Remobilizing fat at such times may dump dangerous quantities of stored contaminants into the blood. What the Dutch team observed may therefore understate the potentially acute threat that chronic ingestion of pollutants might pose to animals under stress, Ferrick says.

Moreover, he points out, Osterhaus' group assayed immune function in these pinnipeds using tests developed for humans and laboratory animals. While they are the existing gold standard, those assays may be relatively insensitive for gauging the health of marine mammals, Ferrick believes.

It's for this reason that Ferrick's team has been working to develop diagnostics for individual components of marine animals' immune systems. Eight to 10 such tests – not only for harbor seals but also for dolphins – are undergoing final refinements. Other assays, for killer whales and sea otters, are in the works.

"If another *Exxon Valdez* accident happened tomorrow, we could already do a much better job [of characterizing its effects on the immune health of marine mammals]" than was possible in 1989, Ferrick maintains. "But what we still wouldn't be able to do is run diagnostics on the blood and do triage – say this animal should be sent for rehabilitation while that one needs to be euthanized." Acquiring this capability may take another 3 to 7 years, he estimates.

For now, Osterhaus and his colleagues continue to closely monitor the immune function of their captive seals. Those once fed Baltic herring now get the same diet of North Atlantic fish as the others. The goal: to see when – if ever – their immune parameters return to normal.

"While this is a very important study in establishing the link between environmental pollution in seals and illness, it's only one study," Shaw says. She emphasizes that there will have to be additional research to prove a causal association. And she'd like to see such studies begin soon, because there may be more than seals at risk.

Because of their large fat deposits and position at the top of the marine food chain, Shaw points out, "all marine mammals are bioaccumulating large quantities of environmental pollutants." Indeed, it's because of these properties, Osterhaus says, that "if any kind of adverse effects were to be expected, then [marine mammals] are the first you'd expect to see them in."

Shaw and Osterhaus note that because humans share that position at the top of the food chain, they also may be at risk. Such concerns haven't stopped Osterhaus from eating fish. However, he says, because the Wadden Sea – the source of fish in his area – is at least as polluted as the Baltic, "we make sure that [its fish] is not a substantial part of our diet."

But limiting fish intake should not be seen as the important take-home message, Osterhaus adds. "If a complete ecosystem is being polluted such that we see effects in its top predators, we have to realize that something is fundamentally wrong here and that we must do something to combat that pollution as it now exists." □

ATTACHMENT #15

State of California - Health and Welfare Agency
PETE WILSON, Governor

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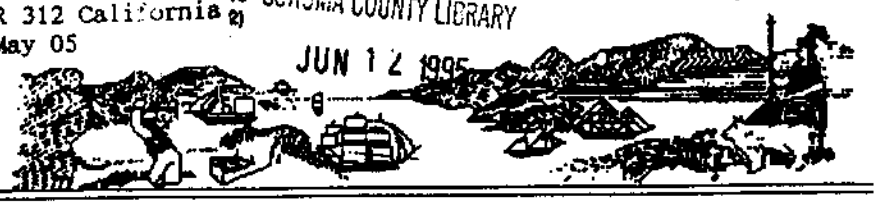
Department of Health Services
S. Kimberly Belshé, Director

California Morbidity

Weekly Report from the
Division of Communicable Disease Control
2151 Berkeley Way, Berkeley, CA 94704-1011
(510) 540-2566 After Hours (510) 540-2306
Contributions are Welcome



CRYPTOSPORIDIOSIS — A GROWING PUBLIC HEALTH CONCERN

Introduction: *Cryptosporidium*, a recently identified human pathogen, has caused at least five waterborne outbreaks of diarrhea in the United States, is commonly fatal in AIDS patients, and is becoming a significant public health issue, particularly because of increasing detections of this parasite in drinking water and the uncertainties concerning the significance of those laboratory findings.

Background on Human Cryptosporidiosis: *Cryptosporidium parvum* has long been recognized as a parasite of a wide variety of vertebrates but it was not noted to cause disease in man until 1976. Since then, it has been identified as a cause of sporadic human gastrointestinal disease and sometimes of outbreaks—the largest being the 1993 waterborne outbreak in Milwaukee in which over 400,000 persons became ill.

The incubation period for cryptosporidiosis is variable, ranging from 2 to 12 days, and averaging 7. The spectrum of illness is also variable, ranging from asymptomatic infection to life-threatening, cholera-like disease, especially in immunocompromised individuals. The major symptoms are abdominal cramps and diarrhea; the diarrhea can be profuse—up to 17 liters/day. Less commonly, there are nausea, vomiting, malaise, and low grade fever. Symptoms tend to wax and wane but remit on their own in 2 to 4 weeks in immunologically healthy people. Due to immunodeficiency, AIDS patients have difficulty clearing their infections; the parasite tends to persist and produce a protracted and sometimes fulminant clinical course that contributes to death. In other groups of immunodeficient persons, such as cancer chemotherapy patients, onset of illness can be explosive with cholera-like symptoms, but the disease tends to last only slightly longer than in otherwise healthy people. There is no specific treatment of reliable efficacy, although octreotide, azithromycin, and paromomycin have been thought helpful in controlling the diarrhea in some patients who were also ill with AIDS. Supportive care via fluid and electrolyte replacement is essential.

Infectivity: The oocysts are considered infective as soon as they are passed in stool and can remain infective outside the body for 2 to 6 months in a moist environment. The infectious dose for cryptosporidiosis infection is low. In one small volunteer study, 20% of those fed 30 oocysts, 38% of those fed 100 oocysts, and 67% of those fed 300 oocysts became infected; the overall illness rate for those excreting oocysts by any infecting dose was 61%. The ID_{50} was calculated as 132 oocysts, a number comparable to that for G. lamblia. In the United States, the background infection rate for *Cryptosporidia* is estimated by the Centers for Disease Control and Prevention at ~1%; in other developed areas, such as Europe, prevalence of infection ranges from < 1% to 4.5%. In developing regions of the world, the prevalence is significantly higher, 3% to 20%.

At increased risk for cryptosporidiosis are child care and health care workers who come in direct contact with feces, diaper-aged children who attend day care centers, persons exposed to human feces by sexual contact, and those (such as veterinarians and animal handlers) with extensive animal contact.

Diagnosis: Diagnosis is made by staining stools. The fewer the organisms, the more difficult the diagnosis. Excretion can be intermittent, and so the parasite may not be detected in every stool. Most excretion occurs within the first 72 hours of infection. Physicians rarely request stool analysis for *Cryptosporidia*, perhaps because (1) excretion is intermittent, (2) laboratory studies can be insensitive, and (3) no reliable, effective treatment exists. A simple, modified Kinyoun stain can be used and is commonly employed. Recently, EIA and FA tests have become available to detect *Cryptosporidia* in stools.

Prevention:

1) Good personal hygiene, by washing hands thoroughly, is critical:
   - after using the toilet, changing diapers, gardening, handling pets or animals, or any possible contact with human or animal feces
   - before handling food and eating
2) Avoid sexual practices that could result in contact with feces
3) Think before drinking:
   - avoid drinking directly from such surface waters as creeks, rivers, and lakes
   - avoid drinking untreated water when travelling in developing countries
   - when unsure of the safety of the water supply, boil water for one minute to kill any *Cryptosporidia* (and other pathogens) that may be present
   - avoid raw milk and raw milk products
4) Those with AIDS and those who are immunosuppressed/immunocompromised for some other reason may want to consider additional precautions to prevent exposure:
   - avoid swallowing water or even immersing the face when swimming
   - avoid contact with animals under six months of age, strays, and animals with diarrhea

State of California - Health and Welfare Agency
PETE WILSON, Governor

May 5, 1995
#17/18
(2 of 2)

Department of Health Services
Kimberly Belshé, Director

California Morbidity

Weekly Report from the Division of Communicable Disease Control
2151 Berkeley Way, Berkeley, CA 94704-1011
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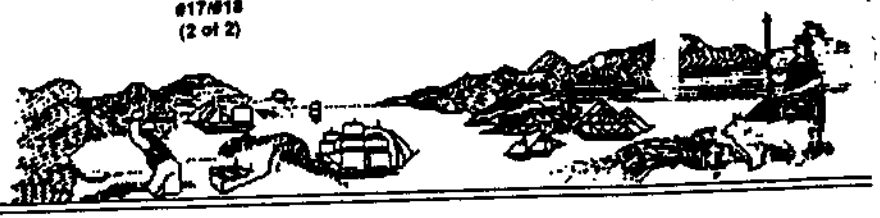


CRYPTOSPORIDIOSIS: CONTINUED

- since the risk of contracting cryptosporidiosis in the absence of an outbreak is unknown, some HIV-infected persons may elect to take independent action to minimize the risk of waterborne cryptosporidiosis. This can be accomplished by bringing water to a full rolling boil for one minute when the water is intended for drinking and/or for making ice. (At elevations greater than 6500 feet, boil for three minutes). When eating out, alternatives to drinks made with cold tap water can be considered.
- other independent actions that can be considered include use of bottled water or home filters, but people should recognize that there are problems in selecting an appropriate product, there are costs involved, enforceable standards do not exist, and that there can be difficulty in using such water consistently for all water that is consumed or used in meal preparation. If home filters are used, only those certified to remove particles less than one micron in diameter by NSF International can reduce the risk of waterborne cryptosporidiosis. Also, there must be strict adherence to the manufacturer's guidelines for proper filter use and cartridge replacement.

Cryptosporidiosis California: Unlike many states where reporting of this disease is not mandated, cryptosporidiosis is a reportable disease in California (since 1989). Annual data: 1989 (since May) - 46 cases; 1990 - 165 cases; 1991 - 210 cases; 1992 - 276 cases; 1993 - 372 cases; and 1994 - 477 cases. Of the 1,546 total cases since 1989, 40.5% were from San Francisco County and 30.8% from Los Angeles County. Of the 1,266 whose sex was reported, 88.4% were males and these were primarily (85.7%) in the 20- to 50-year-old-range, peaking at 30 years. In females, there was a bimodal peak at infancy and at age 30. These statistics are consistent with Office of AIDS data that approximately 4% of AIDS patients are recognized to have cryptosporidiosis. (Cryptosporidiosis is an AIDS-defining condition).

Waterborne Outbreaks: There have been a number of cryptosporidiosis waterborne outbreaks identified in the United States: (1) Milwaukee, Wisconsin; (2) Carrollton, Georgia; (3) Braun Station (near San Antonio), Texas; (4) Jackson County, Oregon; and (5) New Mexico. With the exception of the New Mexico outbreak, all were associated with treated water that met existing EPA coliform and turbidity standards. A sixth outbreak in Las Vegas appears to have been waterborne, too, but findings of that investigation have not yet been published.

Besides its magnitude, the Milwaukee outbreak is important because it involved a municipal supply that had no obvious mechanical breakdown of its flocculators or filters. The Carrollton outbreak was thought to be related to the removal of mechanical agitators in the water treatment plant, leading to decreased efficiency of the flocculation step and impaired particulate removal. Filter efficiency was also decreased by impairment of the equipment that controlled water flow through the plant and the filters were not always backflushed. The city's water supply was drawn from a river that flowed through pasture land where cattle grazed, and stool analysis from cows grazing nearby yielded Cryptosporidia. The Texas outbreak resulted from a sewage-contaminated well. The New Mexico outbreak resulted from drinking untreated surface water (lake water), and some patients acquired Giardia there as well. Among the recommendations issued by the Milwaukee researchers was that water treatment plants should consider instituting continuous monitoring of treated water for turbidity, especially of filtered effluent, and that plant operators should endeavor to reduce turbidity to ≤ 0.1 NTU, a standard that reduces the likelihood of oocysts being present.

Recreational water exposure (chlorinated pools) has also resulted in cryptosporidiosis: in Milwaukee in 1993; in Lane County, Oregon in 1992; and in Los Angeles in 1988.

Issues of Concern: Of all the routes of transmission, waterborne outbreaks cause the greatest concern because of their potential to be extraordinarily large. Chlorine disinfection is not effective: free chlorine concentrations greater than 8,000 mg/L are needed to kill all oocysts; such a dose is not feasible. Even undiluted household bleach (= 5% hypochlorite) is ineffective. Failure of any single phase of a multistage water treatment process can be sufficient to put an entire community at risk, although the recent outbreak in Las Vegas (apparently waterborne) occurred without any identified failure in the water treatment process.

Research Needs:

- improve methods for the isolation, identification, and enumeration of *Cryptosporidia* oocysts in water
- develop methods to distinguish species that are pathogenic to man from those that are not
- develop methods to determine oocyst viability (presently, it is difficult/impossible to know whether oocysts that are detected are viable or not, except by expensive and time-consuming animal assay)
- identify the best water treatment procedures to eliminate/control oocysts in drinking water
- identify modes of transmission and risk of contracting cryptosporidiosis in non-outbreak settings

CALIFORNIA DEPARTMENT OF HEALTH SERVICES - DIVISION OF COMMUNICABLE DISEASE CONTROL - SURVEILLANCE AND STATISTICS SECTION
CALIFORNIA, SELECTED REPORTABLE DISEASES
WEEK 17 ENDING 04/29/1995

DISEASE	CASES REPORTED FOR PERIOD 1995	1994	1993	CASES REPORTED TO DATE 1995	1994	1993
AIDS /1	-	-	-	3124	4625	9395
AMEBIASIS	17	20	17	314	318	394
ANTHRAX	-	-	-	-	-	-
BOTULISM:						
- FOODBORNE	-	-	-	-	-	-
- INFANT /1	-	-	-	2	11	5
- WOUND	-	-	-	4	6	1
BRUCELLOSIS	6	-	-	8	8	5
CAMPYLOBACTERIOSIS	81	179	110	2163	2021	1850
CHANCROID/1	-	-	-	4	14	51
CHLAMYDIAL INFECTNS.*	538	870	830	17295	25388	22956
CHOLERA	-	-	-	2	2	3
COCCIDIOIDOMYCOSIS	19	52	29	349	1307	1675
CONJUNCT. NEWBORN	-	-	1	22	16	17
CRYPTOSPORIDIOSIS	7	8	4	120	135	98
CYSTICERCOSIS	3	2	-	40	48	47
DENGUE	-	-	-	4	2	2
DIARRHEA, NNB. O/B	-	-	-	-	-	-
DIPHTHERIA	-	-	-	1	-	-
ENCEPHALITIS:						
- ARBOVIRAL	-	-	-	-	-	-
- PRIMARY & OTHER	1	5	2	26	35	42
- POST-INFECTIOUS	-	1	-	1	4	10
E. COLI (0157:H7)	1	-	1	19	14	10
FOODBORNE ILLNESS:						
- OUTBREAKS	-	1	-	13	17	15
- CASES	-	3	-	377	419	227
GIARDIASIS	87	104	97	1627	1990	2177
GONOCOCCAL INFECTNS*	160	254	298	7549	9247	10317
GRANULOMA INGUINALE/1	-	-	-	-	-	1
HAEMOPHILUS INFLUZ.	4	1	2	76	43	83
HEPATITIS:						
- TYPE A	156	115	99	2226	1969	1748
- TYPE B	45	67	32	610	712	739
- TYPE D	-	-	-	-	1	3
- NON-A / NON-B	15	10	14	151	159	233
- UNSPECIFIED	3	2	-	55	51	68
KAWASAKI SYNDROME	-	1	7	40	38	91
LEGIONELLOSIS	8	3	-	40	20	24
LEPROSY	-	1	1	23	12	31
LEPTOSPIROSIS	-	-	-	-	-	-
LISTERIOSIS	2	3	4	39	32	33
LYME DISEASE	1	2	8	17	19	50
LYMPHOGRANULOMA VEN/1	-	-	-	1	5	4
MALARIA	2	5	3	51	75	87

DISEASE	CASES REPORTED FOR PERIOD 1995	1994	1993	CASES REPORTED TO DATE 1995	1994	1993
MEASLES:						
TOTAL	1	1	-	83	9	4
- INDIGENOUS	1	-	-	82	7	3
- IMPORTED	-	1	-	1	2	1
MENINGITIS, VIRAL	19	31	27	295	292	542
MENINGOCOCCAL INF.	3	4	8	151	139	133
MUMPS	2	6	6	76	77	100
N G U *	27	121	121	1977	2274	2953
PERTUSSIS	10	14	5	108	152	131
P I D *	11	18	40	781	616	933
PLAGUE	-	-	-	1	1	-
POLIOMYELITIS	-	-	-	-	-	-
PSITTACOSIS	-	-	-	5	2	1
Q FEVER	-	-	-	-	2	1
RABIES:						
- ANIMAL	1	8	8	97	75	134
- HUMAN	-	-	-	-	-	-
RELAPSING FEVER	-	-	-	1	-	1
REYE SYNDROME	-	-	-	-	4	-
RHEUMATIC FEVER	-	-	-	3	4	9
ROCKY MTN SPOT FVR	-	-	-	-	-	-
RUBELLA	-	-	-	9	13	15
RUBELLA, CONGENITAL	-	-	-	3	2	2
SALMONELLOSIS	95	127	112	1530	1479	1855
SHIGELLOSIS:						
TOTAL	31	59	66	996	1059	1115
- GROUP A	-	-	-	13	17	10
- GROUP B	15	19	20	422	396	434
- GROUP C	-	1	2	19	13	23
- GROUP D	11	23	36	400	471	490
- GRP. UNSPECIFIED	5	16	8	142	162	158
SYPHILIS: /1						
TOTAL	-	-	-	1200	2593	3586
- PRIMARY	-	-	-	55	135	190
- SECONDARY	-	-	-	90	154	258
- EARLY LATENT	-	-	-	329	659	1082
- LATE & LATE LTN.	-	-	-	697	1512	1939
- CONGENITAL	-	-	-	29	133	117
TETANUS	-	-	-	2	1	-
TOXIC SHOCK SYNDROME	-	-	-	13	19	12
TRICHINOSIS	-	-	-	1	10	1
TUBERCULOSIS	61	180	71	1079	1692	1528
TULAREMIA	-	-	-	-	-	1
TYPHOID FEVER	3	2	4	19	19	37
TYPHUS FEVER	1	-	1	3	11	1
VIBRIO INFECTIONS	1	-	1	7	7	3
YELLOW FEVER	-	-	-	-	-	-

/1 Reported monthly only. See monthly summary.
* Reports for Los Angeles and San Francisco, updated monthly. See monthly summary.

(provisional data, report generated 05/11/95)

The following is a confidential personnel record of the City of Santa Rosa. The names of personnel named within the record have been removed to preserve the privacy of such personnel.

ATTACHMENT #16

CITY OF SANTA ROSA
UTILITIES DEPARTMENT
M E M O R A N D U M

DATE: July 11, 1994

TO: [redacted]

FROM: MILES FERRIS/SCOTT STINEBAUGH

SUBJECT: SUE KRAMER REPORT/RECOMMENDATIONS

As you are all aware, there has been a considerable amount of unrest and dissention among employees at the treatment plant over the past year or so. We have looked into several ways to make this work place better, the latest of which was a possible intervention effort by Sue Kramer. Sue completed the first step of this effort, which was an information gathering effort to determine if her intervention would be appropriate. Her report is attached for your review.

An issue that has been a constant topic of discussion is when, or if, [redacted] would return to [redacted] position of Plant Superintendent. In order to stabilize the situation and put this issue to rest, we have determined that [redacted] will not return to [redacted] previous position for at least two years from the date of this memo. After that time has elapsed, any assignment will be made based on what will best achieve the goals of operating the plant in the most effective manner, and providing the most positive work environment for all employees.

The time has come for all of us to put past problems in the past, and to move ahead with creating a working environment that is positive and supportive of each other. We must all open ourselves up to constructive criticism, and not tolerate destructive criticism, and we must all treat each other with respect. Management recognizes that we have not been as open to employee input as we might have been, but we are committed to do so in the future. This will not happen instantly; therefore, we ask for your patience and assistance as we move ahead with making the changes that are necessary.

What follows in this memo is our attempt understand what you have told Ms. Kramer and others, and to make positive changes in the way we manage, the way we interact with each other, and the way we go about making decisions.

Ms. Kramer's report contains references for the need to develop communication skills, to develop conflict resolution skills, and to listen to individual concerns. We believe that it is critically important that we all become involved in the solution to these concerns and issues. We suggest that the issues can only be solved by the operators willing participation in the problem resolution and by their participation in the recommended solutions. We intend to establish a committee of operators, representatives from each shift, selected by peers, senior operators, and management. The Committee will suggest a course of action and develop a plan to address the concerns and various other issues which exist. The Committee will be selected and begin meeting before July 31, 1994.

[illegible] rt/Recommendations
Continued -

Ms. Kramer's report makes twelve suggested recommendations which are addressed in order below: These paragraph numbers correspond with the numbering beginning on Page 3 of her report.

1. The City has invested a good deal of time and effort toward improving communication and giving and receiving feedback through the Working Program. Many, if not most of the operators, have not yet attended the Working Program. We intend to accelerate our efforts to schedule the remaining employees in this worthwhile program. Employees who work during the off hours will be rescheduled to work on relief shift while attending the Working Program. Since this involves a shift schedule change, the union will need to be consulted.

2. A part of the City of Santa Rosa's new management evaluation process emphasizes management's responsibility to promote and model the Organizational Values and Basic Principles. This will be given a strong emphasis in our semi-annual performance review of each manager's work plan. We also intend to work with [illegible] of the Personnel Department to develop a special training program for managers which will include developing communication skills. The training plan will be developed by Personnel and all managers will be trained by September 1, 1994.

3. Interpersonal relationship skill building for staff will be addressed by utilizing the skills that management or non-management learn from participating in the Working Program and by observing the behaviors their Supervisors will be expected to model. Staff will be tasked to become competent in this non-technical aspect of our work place. The employee's evaluations will include a review of how well each person maintains constructive relationships with their co-workers, managers and subordinates.

4. The Sue Kramer report, as well as this memo, will be distributed to staff as she has recommended. The rebuilding of trust will occur if we follow through with what we are committing to do.

5. Staff members who treat others with respect will have their efforts reinforced and commended. Those who do not act respectfully will be held accountable for their acts. This applies to managers as well as non-managers. Employees will be recognized for their achievements in their evaluations and at regular staff meetings throughout the year. Input from the operators and the Operators' Committee will be sought out for these evaluations and recognitions.

6. Empowering staff and delegating authority will be emphasized. Several work groups have been empowered in the recent past to address issues of operator concern. These groups will be encouraged to continue their work. A new task will be assigned to the Operator's Committee to work on a system of rewards to give recognition to their co-workers for extraordinary achievement.

Sue Kramer Report/Recommendations
Continued -

7. Management is committed to meeting with the union and staff to address the issues of shift assignments and support for night workers. This effort is underway at this time.

8. The article Sue refers to in her report is attached for your information.

9. The management team of the Utilities Department will develop and strengthen their commitment to proper use of the chain of command. Management will also institute a process to redirect attempts to circumvent the chain of command by supporting and not supplanting those above and below them. The Open Door policy will not be eliminated but the focus will be to redirect attempts at an end run back to the appropriate point in the chain of command. This includes a commitment from Utilities management as well as Personnel and the Manager's Office. These items will be made a part of the management work plans.

10. The issue of who trains the trainees was addressed when the Operator Training Program was created which delegated that responsibility to the Senior Operators.

11. We strongly agree with the recommendation that positive reinforcement should be given to the efforts of the many dedicated operators who want to leave dissension behind and begin to strengthen the operator work team. We must leave the past behind and eliminate threats and perceptions of threats by both managers and staff.

12. There are currently several different committees at work to address issues of concern. One such committee is the Subregional Discussion Team. A redefining of this committee's role and including union and management membership will provide for the "safety valve" or internal audit committee. The Discussion Team will then review and comment on the Operations Committee suggestions. This will provide a check by those who have an outsiders view of the situation and can provide an impartial opinion of the undertakings.

All of this is a significant undertaking, and it will not be easy for any of us, but together we can make it happen. Again, we ask for the positive cooperation of all of you as we move to the future and toward a better place for all of us to work.

Attachment:

P\KRAMERPT.F

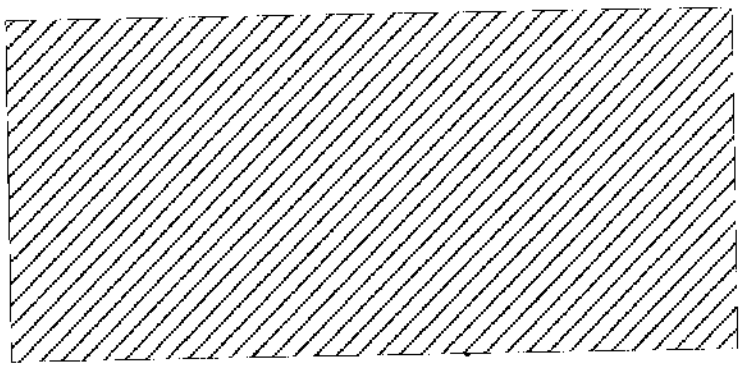
REPORT
VISITS TO SANTA ROSA WASTEWATER TREATMENT PLANT
by Sue Kramer, Consultant

Purpose: to determine whether I would be able to resolve ongoing interpersonal problems at the plant by facilitating a mediation among staff representing various points of view.

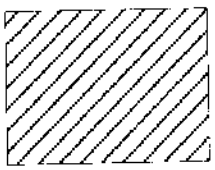
Method: Twelve representatives of various points of view were selected for interviews with me. A memo announcing the interviews was sent to all hands and those not scheduled to talk with me were invited to write their answers to the following questions:

1. "What do you see as the current problem at the plant?"
2. "How is it affecting your job and your work life?"
3. "For you, what would a better work environment look like?"

I conducted two-hour interviews with twelve people and received written responses from five more. Plant Staff interviewed:



In addition, I received written responses from:



Observation: I was greatly impressed by Wastewater Plant Staff members whom I interviewed and those who took the initiative to compose written responses. All of them exhibited a high degree of professionalism, pride in their work and concern for maintaining operations of outstanding quality. Their areas of disagreement relate to interpersonal issues: the way people are managed and the ways they deal with each other.

Conclusion: On the basis of the information I obtained, I think it unlikely that resolution of "the current problem at the plant." would be achieved by my conducting a mediation among conflicting parties at the present time.

Reasons for Conclusion:

1. There is no universal definition of "The problem" as is evident from the following selection of answers given to question #1:

"The problem is a lack of honest communication."
"The problem is that people are out to get[redacted]"
"The problem is that[redacted] is out to get us"
"The whole problem was caused by people ignoring the chain of command"
"The problem is that the chain of command is useless"
"The problem is that things aren't out in the open; information is withheld"
"The problem is the graveyard shift; it's isolated; people don't get complete information."
"The problem is one-way communication. Management thinks everything is fixed once they give an order or distribute a memo."
"The problem is a total lack of trust."
"One big problem is[redacted] not approachable;[redacted] dwells on negatives, not positives."
"The problem started when[redacted] didn't get the job and[redacted] did. There was resentment between[redacted] and[redacted] after that and[redacted] kept everything from going to[redacted]"
"The problem is Utilities management, period.[redacted] is a politician;[redacted] tells people what[redacted] thinks they want to hear but[redacted] doesn't do anything to fix problems."
"The problem is that we used to have a great family atmosphere but[redacted] ruined it by messing with a subordinate and then lying about it"
"The problem is all this harassment and diversity stuff making white males automatically guilty.
"The problem is that everybody feels betrayed.[redacted] feels the operations staff sold[redacted] out; they feel[redacted] sold them out."
"The problem is the people who keep stirring the pot. Everybody else is sick of it. Management should yank their chain."
"The problem is that we've complained but nothing has been done; nobody has been punished."
"The problem is that nothing has been proved so this thing can't be laid to rest."
"For myself and some of the other operators on the low end of the seniority list, the biggest ongoing problem is seniority bid for shifts each year."
"The problem is people spreading rumors and lies."
"The problem is people's inability to communicate and resolve conflicts"
"The problem is that people are afraid to disagree; they get ostracized."
"The problem is fear of retalliation. A lot of people think[redacted] will get even with them if[redacted] ever gets the chance."
"The problem is politics. Utilities management equals bureaucratic damage control."
"The problem is discord in our operations staff; we have "camps" instead of our former tight-knit group
"The problem is a fragmented union relationship; a few people didn't get the answer they wanted so they went around the union rep."
"The problem is favoritism."
"I'm worried about my job security."
"The problem is that the plant is going through growing pains; our jobs are being constantly redefined. A lot of people are dislocated by change."
"Many people are bitter about how they were treated last year."
"Some Operators fear the inevitable confrontations which will occur when people begin working together again."
"Some people mistrust Management for failing to prevent or respond promptly to the problems of the past."

2. There is no agreement on the seriousness of "the problem" (Question 2). While most people described the current plant environment as more than optimally stressful, there were vast differences in perception of the intensity, scope and criticality of the situation. Opinions ranged from "Things happen. We should just let go and move on," to "The City better do something or this whole place will blow up."

3. There is insufficient faith that mediation will help at this point. Some said they'd be willing to try, but they expressed skepticism about the efficacy of "any more talk." A small but vocal group has an intense emotional opposition to any attempt to solve "the problem" through mediation. For a variety of individual reasons, these Operators are fixated on preventing [redacted] return to the plant. They vehemently oppose any intervention which involves building a bridge between them and [redacted], fearing that [redacted] could then walk across the bridge and return.

4. "The problem" is not a single issue amenable to a single solution strategy. Given its amorphous nature, a direct assault on "the problem" would be like fighting a cloud. "The problem" is a state of unrest, variously perceived and interpreted. It is symptomatic of a number of underlying issues, each of which should be specifically addressed. In fact, several Operators told me that they regarded the [redacted] situation as a catalyst which brought to light some problems which had been around for a long time.

Issues and Recommendations: Listed below are some of the issues which surfaced in my conversations and a few recommendations for dealing with them:

1. Communication. The Utilities Department must improve communication channels up, down and across. They should develop and use effective systems for giving clear, objective and timely information and should train managers and staff in communication skills, including giving and receiving feedback. Special attention should be paid to making sure individual concerns are heard, to periodic checks on the effectiveness of the system and to information transfer across shifts.

2. Management training. In the Utilities Department, people responsible for managing people need extensive training in people-development skills such as coaching, delegating, goal setting, performance monitoring, giving and getting feedback. Their skill in managing and developing human resources should be a significant part of their performance reviews and their subordinates should be encouraged to provide input.

3. Skill building. Treatment staff are justifiably proud of their technical skills and professional competence. They need to become equally competent in dealing with the non-technical problems that face them. They should build skills in such areas as communicating clearly, resolving conflicts and dealing with change.

4. Rebuilding Trust. Rebuilding trust takes time and work. Management must begin in small specific instances to say what it means and to mean what it says. Open communication is needed to dispell rumors. Start by disseminating this report or a version of it. Let staff see that management cares about their well-being and is working to address their concerns. Invite their suggestions and create systems for responding to suggestions.

5. Respect for individuals. A workplace where diversity is valued and harassment avoided is not produced by threats but by building a culture of respect. All staff members want and deserve to be treated with respect. Respect for individuals must be taught, cultivated and modeled. Many staff members treat others with respect. Their efforts need to be reinforced, commended and copied.

6. Empowering Staff. People who see themselves as having little power often use negative behaviors to gain a feeling of importance. Giving them real power is a constructive way to end the negative behavior and to reap the benefits of the positive contributions they can make. Supervisors must begin to give workers real power to make decisions, to solve problems and to find better ways to do things. Managers must learn to delegate authority appropriately and, when workers are asked to make a decision, to accept their decision, let them try it and learn from it.

7. Shift Assignments Although permanent shifts are more desirable and healthful than rotating shifts, strong resentment boils up when a low seniority person sees no hope of escape from an undesired permanent assignment. In addition, there are unsolved problems of alienation with staff permanently assigned to work at night. Union, staff and management should work together to assess and find creative solutions to the current problems with shift assignments.

8. Support for night workers. I am attaching an article about the special problems of night workers and what some organizations are doing to support them. Union, management and some night workers should work on this issue. Management should occasionally appear at the plant at 3:00 A.M. with good news and words of praise and encouragement. Meetings should be scheduled with consideration for the sleep times of swing and graveyard shift workers.

9. Management structure/chain of command. The chain of command in Utilities needs to be defined and strengthened. Each level of management and supervision needs to know what they're accountable for, what they should delegate and where their authority ends. Their roles and responsibilities must be clearly defined, including specific goals and objectives, performance measures and checkpoints. In Operations, Senior Operators need to exercise more power.

Staff needs to know to whom to go and for what. They need to know the chain of command, the appropriate information channels and what alternate pathways to take if they encounter blockages. They also need to know what pathways are inappropriate and why. When someone uses an inappropriate pathway, they should be redirected to an appropriate one. An end run around the chain of command is a signal that a link isn't functioning properly. The receiver of the complaint should take immediate action to find out what's wrong with the link and to help to get it fixed. Supervisors must develop and support, not undermine and supplant those below and above them.

10. Trainees. Trainees should be trained by Seniors and Operators, not the Plant Superintendent.

11. Laying the Demons to Rest and Moving forward Along with announcing [redacted] placement, Utilities Management needs to make a definitive statement that it's over. They should declare that as of now, we're putting the past behind us and focusing on the future.

Management should give positive reinforcement to the efforts of the many dedicated operators who

want to leave dissension behind and begin to build the future. Utilities Management might consider putting some energy behind a "We're Moving Forward" campaign. This cannot be seen as an attempt to gloss over peoples' concerns; rather the emphasis should be on learning from our mistakes and strengthening ourselves to prevent repetition of them.

12. Internal Audit Committee. To ensure that we learn from our mistakes and prevent their recurrence, the Utilities Department and the union should consider setting up an internal audit committee with rotating membership representing a variety of levels and assignments. In addition to providing ongoing monitoring and a "safety valve" for internal communications, this committee could gather and disperse information and lead a movement toward continual quality improvement. There is a lot of talent and potential leadership among Wastewater Staff. This committee could assist in developing and using it fully by creating an opportunity for individuals to work together, to contribute their individual expertise and to gain a "big picture" view.