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PREMATURE PARTURITION IN THE CALIFORNIA SEA LION

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Abstract: Twenty percent of the California sea lion pups born on San Miguel Island die due to premature parturition. Specimens collected from premature-partus animals resulted in recovery of a virus, San Miguel Sea Lion Virus, indistinguishable from Vesicular Exanthema of Swine Virus, and Leptospira pomona from some of the premature cows and pups. The age range of 10 females delivering healthy pups in June was 10-14 years. With one exception, the ages in 10 aborting females was 6-8 years. The p,p'- DDE levels of the premature parturient cows' blubber and liver were 7.6 and 4.8 times greater, respectively, than corresponding tissue concentrations in the full-term animals. Polychlorinated biphenyls residues were 4.4 and 3.8 times greater in aborting animals' blubber and liver than in the same tissues of full-term sea lions. Premature-partus females had tissue imbalances of mercury, selenium, cadmium and bromine. Pathology, parasitology, serum enzyme and hormone results are also presented. These data suggest an interrelationship of disease agents and environmental contaminants as the cause of premature parturition.

INTRODUCTION

The beaches at the west point of San Miguel Island, one of California's Channel Islands, are the most northerly and one of two major pupping and breeding grounds for the California sea lion (Zalophus californianus californianus). Pupping and breeding also occur on other Channel Islands as well as on islands off the Pacific and Gulf coasts of Baja California, Mexico. Premature parturition in the San Miguel sea lion population was first observed by one of the authors (R. L. D.) in 1968. Premature births have

been reported as early as January on San Nicolas Island¹⁶ and in February on San Miguel Island.⁶ The birth rate begins to rise from the time of these early observations until the peak of normal pupping in June. However, over a period of just a few days at mid-May the percentage of pups surviving for one week or more changes from 0 to nearly 100.

All premature pups observed from 1968 through 1971 were born alive; subsequent observations by the authors, however, revealed an occasional stillborn pup. Pups born prior to March were not furred, had almost no motor activity other

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than shallow breathing, and lived only minutes to a few hours. Some fur was present on the pups born in March and by late April pelage was similar to full-term pups. These weak pups are distinguished by difficulty in breathing, lack of coordination, and short survival time. They are present through mid-May at which time normal pups, comparable in length and pelage are also present.

A study in 1970° revealed that premature parturient females had tissue concentrations of polychlorinated biphenyl compounds (PCB's) and total DDT compounds (p, p'-DDT + p, p'-DDE + p, p'-DDD) 2.4 to 8.0 times higher than females giving birth to apparently normal pups at full term.

Organochloride compounds are reported to cause reproductive failure in fish,3 birds, 18,19 mink, 9,22 rabbits, 11 mice, rats and dogs. Levels required for impairment of reproduction vary considerably from species to species and have been reported to be reached by fish3 and birds19 in nature. The mechanisms by which reproductive processes are affected also vary considerably. In fish the critical stage is during the absorption of the yolk sac at the fry stage. In birds, reproductive failure due to thin eggshells has been well documented for DDE,19 whereas PCB's affect the embryo.³⁰ Studies on mink^{9,22} indicate this species is more susceptible to DDT and PCB's than laboratory rats and mice.

Chlorinated hydrocarbons induce hepatic microsomal enzyme activity, resulting in an increased rate of hydroxylation of the steroid hormones, including progesterone and estradiol. This reaction makes these compounds more water soluble and, therefore, prone to excretion. If feedback mechanisms do not maintain adequate concentrations of circulating hormones, the result may be termination of pregnancy. The enzyme-inducing capability of these compounds are additive and PCB's, on a weight basis, have about five times the estradiol biotransformation potential of p, p'-DDE.²²

Boars fed sufficient PCB (a total of 100 mg Aroclor 1254 in a single dose or in ten 10 mg doses) to cause significant reduction in urinary estrogen and dehy-

droepiandosterone did not have any visible lesions either at necropsy or on histologic examination.²¹ This illustrates that PCB compounds can cause significant biochemical changes in the absence of demonstrable histopathology.

In addition to induction of microsomal enzyme activity, serum transaminase levels also have been reported to be elevated following PCB exposure. A rise in serum glutamic oxaloacetic transaminase (SGOT) has been reported in rabbits³² and rats² after acute exposure to PCB's (single oral dose Aroclor 1242 100 mg/kg) indicating some liver damage occurred.

Although these examples illustrate a possible direct effect of these pollutants on the reproductive system, the more subtle mechanism of immunosuppression may also be operative. Fish10 and birds8 fed PCB's have shown increased susceptibility to disease. Antibody production in rats decreases as much as 30% after oral exposure to p, p'-DDT, and the lowered serum globulin concentration was accompanied by loss of lymphoid tissue in the spleen.83 In addition to this apparent decrease in the net volume of the reticuloendothelial system, these investigators suggest that p, p'-DDT may inhibit gamma globulin synthesis in the antibody forming cells by interfering with feedback control mechanisms. PCB's fed to guinea pigs at as little as 10 ppm for eight weeks caused significant reduction in gamma globulin containing cells in lymph nodes and in the serum gamma globulin level.31

Leptospirosis was discovered in young male California sea lions along the coast of northern California in the fall of 1970. Serologic tests for antibodies to Leptospira spp. and Brucella abortus were negative for females sampled in 1970 on San Miguel, and routine bacteriologic cultures of uteri of six aborting animals were not significant.

Prior to the 1972 sea lion pupping season we designed a study to confirm the association between high organochlorine residues and premature parturition and to investigate the possibility that microbial agents also may be contributing to the prevalence of abortion in the sea lion.

MATERIALS AND METHODS

California sea lions were selected from beaches at the western point of San Miguel Island from Point Bennett to Adams Cove. Recent parturition was determined in the field by the presence of fresh blood on the female, the condition of the umbilical cord and the presence or absence of a placenta (placentas were usually removed rather rapidly by the Western Gulls, Larus occidentalis, in the area).

Five premature parturient females and their pups were collected between 27 and 29 March, and five between 25 and 27 April. Ten full-term parturient females and their apparently normal pups were collected between 13 and 15 June as controls (Table 3).

A necropsy was performed on each of the twenty females and all but two of the pups. Samples of brain, lung, liver, heart, pancreas, kidney, adrenals, uterus, intestine, spleen and lymph nodes were preserved in 10% buffered formalin for histopathologic examination. A canine tooth was removed from each female to section and determine age. 25,34 Blood was collected from all animals by cardiac puncture within minutes after death and centrifuged in the field. Serum and plasma samples were frozen for serologic studies and for enzyme and hormone assays.

Hormone radioimmunoassay was conducted on 2.0 m of serum extracted with 20 ml of diethyl ether after approximately 1000 c/m of labeled steroid was added to calculate recovery. Separation of steroids was carried out on a Sephadex column (LH-20) using the solvent system isooctane: benzene: methanol, 62:20:18 (V/V). The steroid fractions were assayed using anti-serum from New England Nuclear.

Tissues collected for toxicology included blubber, muscle, liver, kidney and brain from adults and pups. These tissues were wrapped in aluminum foil and

frozen on dry ice in the field, then maintained at -18 C until time of analysis. Subsamples were taken from blubber and liver then homogenized and ground with anhydrous Na₂SO₄. Samples were extracted for a minimum of 8 hr in a Soxhlet apparatus with nanograde hexane. A sulfuric acid/fuming sulfuric acid clean-up (Davidow) was used for all samples. These samples were analyzed using a Tractor MT-220 gas chromatograph equipped with Niss electron capture detectors. Detector, inlet and column temperatures were maintained at 300 C, 225 C, and 180 C, respectively. A 3% OV-1 column on 100/120 Gas Chrom Q was used as the standard column. Samples were saponified to confirm p, p'-DDD and p, p'-DDT. The PCB profiles most closely resembled Aroclor 1254. The PCB peak with retention time 1.48 relative to p, p'-DDE was used for PCB quantification.

Subsamples of liver and kidney were also processed for mercury, selenium, cadmium, silver, bromine, copper, iron, zinc, manganese, potassium, sodium, calcium and magnesium determinations. Two atomic absorption techniques and x-ray florescence were used in these determinations. ¹⁶

Serum glutamic pyruvic transaminase (SGPT) and ornithine carbamyl transferase (OCT) were measured in all 20 females using standard clinical techniques.9

The procedures describing virus isolation techniques, *Leptospira* culture methods and the serological tests used to measure humoral antibody to these organisms have been described.

9

RESULTS

Dead pup counts made in the study area over a five-year period are given in Table 1. The number of these animals recovered at a particular time can be greatly influenced by wind and tide conditions, yet these figures indicate that a

New England Nuclear, Boston, Massachusetts.

Bio-Science Laboratories, Van Nuys, California.

TABLE 1. Census of premature pups, Point Bennett area, San Miguel Island.

Year	Date	Dead	Live-Moribund	Total
1970	25 April	242	N.D.	242
1971	18 May	348	N.D.	348
1972	27 April*	262	4	266
	20 May	539	197	736
1973	11 April*	67	0	67
	18-21 May	432	148	580
1975	23 April	62	0	62
	15 May	228	39	267

Dead pups counted in April of 1972 and 1973 were collected into piles on the beach to avoid the possibility of counting the same animals again in May.
 N.D. = Not Determined.

conservative estimate of pup mortality prior to mid-May may be as high as 20% of the total number of pups born (R. L. De Long, unpublished data).

The age range of full-term parturient females was 10 to 14 years with a mean of 12 years. Ages of aborting females ranged from 6 to 8 years with the exception of one animal whose age was determined to be between 13 and 14 years, bringing the mean age for these 10 females to eight years (Table 3). There was a significant difference (p=.01, Wilcoxon 2-sample test) in the ages of premature parturient females collected in March and those in April, and the age difference between all aborting females and the full term group was also significant (p=.003, Wilcoxon 2-sample test).

Necropsy of the 10 full-term pups failed to reveal any significant lesions, nor were any observed on microscopic examination of the tissue. Females giving birth to normal pups did have some lesions; however, these appeared to be induced by metazoan parasites. Contracaecum osculatum was observed in the stomach contents of most of the animals and some had minor ulceration of the stomach wall. Four of these females (M-11, M-12, M-15, M-18) had a few small biliary granulomas. These lesions appeared

to be caused by the trematode, Zalophotrema hepaticum, in infestations ranging from mild to severe.

Endometrial edema was observed in all the parturient females. Large numbers of microfilariae from *Dipetalonema oden*hali were observed in scrapings of the uterine epithelium.

Non-parasite induced pathology was observed in two of the normal parturient females. One (M-20) had a few small areas of focal interstitial nephritis and the other (M-19) had hypertrophy of the adrenal medulla and edema in the interstitial and peribronchial portions of the lung.

Four of the 10 premature parturient sea lion females also had some liver lesions caused by Zalophotrema (M-4, M-5, M-6, M-10) and most had a slight degree of gastric ulceration due to Contracaecum. A focal interstitial nephritis was noted in three females of this group (M-2, M-4, M-8) and a mild bronchitis or bronchopneumonia was observed in four (M-1, M-4, M-6, M-9). One of the animals with an apparent mild bronchitis (M-9) also had hyperemic tonsils containing pus in the medullary portions. Another female (M-2), in addition to interstitial nephritis, had a congested spleen with an abcess in the central portion.

Lesions in the premature group of pups were of no significance with the exception of two animals. Leptospira pomona was recovered from the placenta and fetus of one aborting female (M-8).27,20 The fetus had a friable liver, subcapsular hemorrhage of the liver and both kidneys and unclotted blood in the peritoneal cavity. Similar lesions were found in another fetus (M-2), however, L. pomona was not recovered. Four aborting females had antibody titers to L. pomona, including the one with interstitial nephritis that shed viable L. pomona in the placenta (Table 2). One animal (M-18) which delivered an apparently normal pup at full term also had a low titer to L. pomona. Virus isolates were obtained from three of the aborting females and from the fetus of one.28,29 Physio-chemical, morphological, and animal infectivity studies revealed these were three distinct serotypes of San Miguel Sea Lion Virus (SMSV), a calicivirus indistinguishable from Vesicular Exanthema of Swine Virus (VESV). Serologic tests detected activity to at least one of the SMSV serotypes in each of the 20 adult females (Table 2).

DDT and PCB tissue residues for blubber and liver are given in Table 3. The p, p'-DDE levels of premature parturient females' blubber and liver were 7.6 and 4.8 times greater, respectively, than corresponding concentrations in full-term animals. PCB residues were 4.4 and 3.8 times greater in aborting animals' blubber and liver than in the same tissues of full-term sea lions. (Mean data for 19707 and 1972 are given in figures 1 and 2.) Collectively, these data show that the premature parturient females had mean liver p, p'-DDE and PCB residues 4.0 times greater than those animals giving birth at full term. The differences in concentrations of DDE and PCB in both blubber and liver between the two groups are highly significant (p < .001, Wilcoxon 2-sample test). Moreover, the premature parturient females had a higher DDE/PCB ratio than did the full-term parturient females (p < .001, Wilcoxon 2-sample test).

Full-term females and pups had high concentrations and a good intra- and inter-organ balance of mercury, selenium, cadmium and biomine in liver and kidney tissue. Premature parturient sea lions and their pups had lower levels of these elements in imbalanced proportions. Manganese was higher in normal females

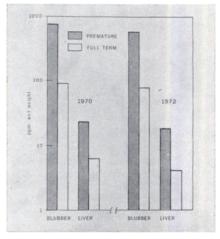


FIGURE 1. Mean tissue residues of p,p'-DDE in parturient California sea lions collected on San Miguel Island.

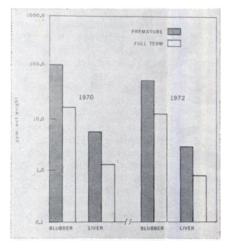


FIGURE 2. Mean tissue residues of PCB's in parturient California sea lions collected on San Miguel Island.

TABLE 2. Leptospira pomona, SMSV isolations and serologic results, liver function enzyme determinations and steroid hormone levels in the adult female California sea lions collected in 1972.

	I nomen			S	SMSV ²			Transa	aminase	Ste	Steroid
	т. ротопа			Titers to	Titers to Serotypesa			Acti	Activity4	Hor	mones
Animal No.	Titer	Isolates	-	7	4	S	Isolates	SGPT	OCT	Estradiol	Progesterone
					Prematu	re Partus	Premature Partus Sea Lions				
M-1	<1:100	ı	1:10	1:80	none	1:10	rectum	34	5.2	N.D.	Z.Ö.
	,						fetus				
M-2	<1:100	I	1:40	1:320	none	1:10	rectum	27	0.7	N.D.	Z.O.Z
							throat				
M-3	<1:100	I	nome	1:160	none	1:10	ı	25	6.0	40.1	70.3
M -4	<1:100	ļ	1:20	1:80	none	1:10	l	78	33.1	35.7	68.3
M-5	<1:100	1	1:80	1:160	none	1:20	i	46	10.6	48.0	74.7
9-W	<1:100	1	1:40	1:20	none	1:20	l	56	0.3	34.0	57.3
M-7	1:400	I	1:10	1:60	none	none	rectum	55	2.6	Z. Ö.	Z.O.Z
M-8	1:640	kidney	none	1:40	none	1:10	ı	35	3.0	28.6	49.8
M -9	1:200	1	1:20	1:80	1:10	1:40	1	45	2.8	Z.D.	Z. Ö.
M-10	1:400	I	1:10	1:80	none	1:10	l	30	1.9	23.4	50.4
					Full-ter	Full-term Partus	s Sea Lions				
M-11	<1:100	I	1:20	1:80	none		I	33	0.5	60.1	93.4
M-12	<1:100	l	1:10	1:40	none	1:10	I	26	3.5	Z.O.	Z.O.
M-13	<1:100	I	1:20	1:80	none	1:10	ļ	36	3.2	8.65	84.8
M-14	<1:100	İ	1:40	1:10	none	none	ı	40	0.2	46.4	70.3
M-15	<1:100	i	1:40	1:40	none	1:20	İ	31	0.3	Z.O.	Z.O.
M-16	<1:100	1	1:10	1:40	none	none	-	27	1.0	Z.O.	N.D.
M-17	<1:100	I	1:10	1:20	none	none	ı	33	0.3	Z.Ö.	Z.D.
M-18	1:100	I	1:10	1:80	none	1:10	ı	4	2.5	49.0	81.8
M-19	<1:100	I	1:20	1:40	none	none	ļ	4	1.6	62.1	71.1
M-20	1:100	į	1:40	1:160	none	none	ı	30	0.9	58.0	8.69

¹ See reference 28.
² Smith, A. W. Unpublished data.
OC
³ Neutralizing antibody titer to SMSV serotypes.

4 SGPT: enzyme activity units. ^a Estradiol: picograms/ml Progesterone: nanograms/ml OCT: nanomoles. ^a Histologic examination revealed intersitial nephritis in kidneys.

TABLE 3. PCB and DDT metabolite tissue levels in premature and full-term partus California sea lions from San Miguel Island, 1972 (parts per million, wet weight).

	Age	Date			Blubber	- Ser			Liver	
Animal No.	(years)	Collected	P	p,p'-DDE	p,p'-DDD	p,p'-DDT	PCB	p,p'-DDE	p,p'-DDD	PCB
				Premai	Premature Partus Sea Lions	a Lions				
M-1	7		72	580	15.1	2.97	49.4	26.6	3.22	4.34
M-2	9		72	879	15.8	5.56	67.4	13.7	0.25	3.10
M-3	7		72	355	11.5	4.13	33.0	8.54	0.034	0.63
M-4	9		72	927	19.7	10.1	57.5	24.1	0.157	1.22
M-5	7	29 Mar	72	350	8.73	5.74	47.1	13.0	0.051	0.77
9-W	œ		72							
M-7	∞		72	633	6.67	4.88	54.1	19.6	0.139	2.53
M-8	13-14		72	375	13.7	11.2	56.4	12.4	0.273	4.13
M -9	∞		72					20.7	0.174	4.35
M-10	7	27 Apr	72	939	18.7	15.8	92.4	30.4	0.91	80.9
				Full-te	Full-term Partus Sea Lions	a Lions				
M-11	13	13 Jun	72	82.6	3.02	3.09	12.1	5.98	0.41	1.86
M-12	10		72	24.1	1.17	1.15	5.19	1.42	0.057	0.43
M-13	10	13 Jun	72	141	2.41	3.67	20.8	4.72	0.063	1.07
M-14	11	14 Jun	72	342	5.55	3.87	39.5	18.2	0.15	1.98
M-15	13-14		72	24.3	1.14	2.06	8.31	0.93	0.044	0.28
M-16	13	14 Jun	72	16.3	1.04	1.17	5.39	1.08	0.061	0.19
M-17	10		72	17.4	0.65	1.84	4.73	1.15	0.028	0.24
M-18	11		72	31.5	2.65	2.26	6.33	1.38	0.041	0.41
M-19	12		72	23.3	1.41	2.53	5.16	1.17	0.064	0.43
M-20	12		72	127	5.50	0.72	24.1	5.54	0.087	1.04

while copper was highest in premature partus animals. Concentrations of the other elements examined were essentially the same in both groups.¹⁴

The results of serum analyses for estradiol and progesterone for six of the 10 females in each group are in Table 2.

Means of the SGPT and OCT values reported in Table 2 did not differ significantly between groups. Female M-4, with the highest SGPT and greatly ele-

vated OCT, had the most severe infestation of liver flukes and associated peribiliary hepatitis and fibrosis, probably accounting for the high transaminase levels.

Table 4 contains the standard lengths' and sex of the pups recovered in this study. There is essentially a 1:1 distribution of males and females in both normal and premature group.

TABLE 4. Sex and standard lengths of pups from California sea lion females M-1 to M-20 collected from San Miguel Island, 1972.

Animal No. *	Sex	Standard Length (cm)**
F-1	M	69
F-2	M	N.D.
F-3	F	N.D.
F-4	F	52
F-5	M	63
F-6	F	61
F-7	N.D.	N.D.
F-8	F	64
F-9	M	67
F-10	F	60
F-11	F	77
F-12	F	74
F-13	F	74
F-14	М	77
F-15	F	70
F-16	M	75
F-17	M	73
F-18	M	73
F-19	M	75
F-20	F	76

^{*} F-1 is the pup from M-1, F-2 from M-2, etc.

DISCUSSION

L. pomona has been identified as a cause of abortion in several animal species²⁴ and was also implicated in the deaths of many subadult and adult male California sea lions in 1970.²⁰ Earlier that same year serum antibodies to Leptospira

spp. could not be demonstrated in six sea lions that aborted on San Miguel Island.⁶ During this 1972 study, however, the organism was isolated from an aborted fetus and lesions consistent with infection by this pathogen were observed. The female giving birth to this pup had a high

^{**} See Reference 1.

antibody titer to *L. pomona* as well as did three other premature parturient animals. Subsequent studies during 1973 resulted in the isolation of *L. pomona* from two additional aborted pups.²⁷ In each instance the pups had extensive multiple hemorrhages comparable to the fetus mentioned above. This demonstrates that some reproductive failures in the California sea lion are associated with *L. pomona* infection.

SMSV was recovered from three of the premature parturient adults and one fetus in 1972. In 1973 two additional isolations were made from throat swabs of two freshly aborted fetuses. Swabs of 28 other fetuses were negative. These isolations, together with neutralizing antibodies to 2 to 4 serotypes of SMSV in most of the adult females in 1972, indicates there is a very high prevalence of this virus in the sea lion population. Vesicular lesions of SMSV have been observed on flippers of Northern fur seals (Callorhinus ursinus) but were not observed in these animals.

A high prevalence of SMSV in the sea lion population is indicated by the serologic findings; reproduction of the older animals, however, does not appear to be affected. Premature parturition in younger animals may be one critical manifestation of SMSV infection in the sea lion.

In addition to isolation of these two biological agents, both associated with reproductive failures in domestic live-stock,²¹ data collected during this study and in 1970 indicate that organochlorine pollutants are associated with premature births. Differences in tissue levels of these compounds between the two groups warrant attention and a practicable explanation for the wide difference has been proposed.⁷

If younger female sea lions winter in the Channel Islands vicinity, they would be feeding on fish containing much higher levels of DDT and PCB compounds than older animals wintering off Mexico. Different DDE/PCB ratios in the two groups also suggest that they have been feeding in different areas.

Significant four-fold differences for p, p'-DDE and PCB's in the liver suggests that these compounds are also a

factor in early termination of pregnancy in the sea lion. Enzyme induction would cause biotransformation of endogenous steroids to hydroxyl derivatives, affecting the reproductive cycle by altering the normal hormonal balance controlling gestation. Steroid hormone levels in Table 2 do not permit a rigorous comparison of serum concentrations between normal and premature parturient females because the animals were sampled at a different time in the gestation cycle. Levels of both estradiol (p=.002) and progesterone (p=.02, Wilcoxon 2-sample test) are lower in the blood of the premature parturient females but these hormone levels are expected to change during pregnancy, therefore the significance of this observation presently cannot be explained.

If immunosupression occurs in sea lions due to high organochlorine tissue concentrations, response of the immune system to an invading pathogen would be tempered. DDT and PCB compounds could therefore induce an abnormal susceptibility to disease producing microorganisms, including those causing premature parturition. Lesions or changes in the respiratory system in nearly half of the premature group and isolation of pathogens known to induce abortion from four of the animals could indicate that their immune systems had been compromised in this manner. The total DDT and PCB compound residues in the livers of the three adults from which SMSV was recovered are distributed equally about the mean levels for the premature group. Total DDT and PCB compounds in the liver of the single female from which L. pomona was recovered were 12.7 and 4.1 ppm, respectively. Conclusions with regard to this mechanism, however, cannot be substantiated without more information.

Mercury, cadmium and selenium accumulate with age and therefore higher concentrations are expected in the normal partus animals. Imbalances of these elements, however, are more important than absolute levels and this condition in premature partus females could have resulted in toxic effects related to early parturition.¹⁴

The age structure of samples of females collected in 1972 confirmed the 1970 observation that younger females are more prone to premature parturition. Early termination of pregnancy due to age alone does not appear likely although in another otariid, the northern fur seal, there is a slightly higher tendency for younger females to abort or resorb the conceptus. Of 3111 female northern fur seals collected at sea between 1958 and 1961 at latitudes between California and British Columbia, 9.26% of females 4 to 8 years old (n=905) had aborted or resorbed and 7.78% of females 9 to 15 years old (n=1837) had aborted or resorbed. 34,15,7 This difference is significant at an alpha level of 0.10, against a onesided alternative, using an arcsine transformation test of equal percentages (p = 0.094). Fur seals do not show the pronounced tendency for only young age classes of females to experience reproductive failure as we observed in the California sea lion. Significant differences in age, pollutant levels and DDE/PCB ratios between premature and full-term parturient female California sea lions indicate that younger females remain and feed in the Channel Islands vicinity for a much longer time than older females, thus accumulating higher tissue organochlorine residues.

Further studies are necessary to elucidate the relative importance and interrelationship of the calicivirus, leptospires and environmental pollutants in the overall phenomenon of reproductive failure among California sea lions.

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