# Premature Births in California Sea Lions: Association with High Organochlorine Pollutant Residue Levels

Abstract. *Premature pupping in California sea lions has been noted on the breeding islands since 1968. Organochlorine pesticides and polychlorinated biphenyl residues were two to eight times higher in tissues of premature parturient females and pups than in similar tissues of full-term parturient females and pups collected on San Miguel Island in 1970.*

The California sea lion (*Zalophus californianus californianus*) breeds on California's Channel Islands and on islands off the Pacific and Gulf coasts of Baja California, Mexico. In the Channel Islands normal pupping occurs between 15 May and 30 June and breeding between 20 June and 20 July. We have observed early termination of pregnancies among animals on several breeding rookeries, including those on San Miguel Island, since 1968. Premature births were reported by Simpson and Gilmartin (*1*) on San Miguel Island and by Odell (*2*) on San Nicolas Island, and were discussed by Brownell and LeBoeuf (*3*). An earlier investigation into the causes of these premature births was inconclusive (*1*). The work reported here was conducted to determine whether environmental pollutant residues may be related to premature partus in the California sea lion.

In the Channel Islands premature births occur as early as January and appear with increasing frequency until mid-May. We observed live premature pups from February through mid-May, and saw no pups which were dead at birth. The early pups are not furred and appear to die soon after birth. The premature pups born in March, April, and early May are furred, but they lack motor coordination, their breathing is short, and only portions of the lungs have been aerated. Late premature pups appear to live from a few hours to possibly as long as several days. There is no apparent temporal break between the end of premature births and the onset of the normal pupping season. About 15 May the death rate decreases and some pups survive. In mid-May both live premature and full-term pups are present; full-term pups are easily distinguished as they have full motor coordination. The quantitative incidence of premature births has not been well established. On San Miguel Island we counted 242 dead premature pups on 25 April 1970 and 348 on 18 May 1971 from an estimated total population of 10,000 to 15,000. On San Nicolas Island, Odell (*2*) reported 442 premature pups from a female population of 5,500 between 17 January and 3 May 1970.

In 1970 we collected two groups of postpartum female California sea lions within 24 hours after parturition on San Miguel Island. The premature parturient group of six females and their premature pups were collected between 20 and 24 April. The full-term parturient group of four females and their live pups were collected between 24 and 26 June, late in the normal pupping season.

Ages of adult females were determined by counting growth layers in the dentine of longitudinally sectioned canines (*4*). The liver, brain, and blubber from cows and pups were analyzed for DDT compounds (*p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE), dieldrin, and polychlorinated biphenyls (PCB's) (*5*). Identification of PCB's was based on the characteristic profiles of peaks on the gas chromatograms obtained with a DC-200 column; quantification was achieved through a comparison of the height of the peak emerging between the peaks for *p,p'*-DDD and *p,p'*-DDT with the height of the corresponding peak in chromatograms of the standard (Aroclor 1254, Monsanto Co.) (*6*). Blubber and liver tissue from females were analyzed as they represent a storage and metabolism site for organochlorine residues. Brain tissue from pups was analyzed to gain some insight into the behavior of the premature pups. Organochlorine pesticide and PCB residues were found in all tissues collected and tested (Table 1). The most prevalent metabolite was found to be DDE (80 to 93 percent of the total DDT).

All analyzed tissues from females which gave birth prematurely in 1970 contained higher concentrations than the tissues from females which gave birth to full-term pups. The mean total DDT residues in the blubber and liver of premature parturient females were 8.0 and 3.8 times greater, respectively, than the concentrations in the same tissues of normal parturient cows. The brains of premature pups contained twice the residue concentrations as the brains of full-term pups. The PCB levels in blubber, liver, and brain of the premature parturient group were 6.6, 4.4, and 2.4 times higher than those in the full-term group. In neither blubber nor liver did the ranges of residue values of total DDT or PCB in premature and full-term groups overlap (Table 1). Dieldrin residues were not detected in all samples and when present, were low. Figure 1 is a histogram of the DDT and PCB residues in the two groups of animals, showing the relative differences in tissue pollutant concentrations.

Histological sections of liver tissue revealed normal tissues from all prema-

Table 1. Concentration of pollutant residues in tissues of parturient California sea lion females and pups, in parts per million (ppm), wet weight; *N*, number of animals; $\bar{X}$, mean of two (brain, liver) and three (blubber) residue determinations on each tissue of each animal. The age range is 6 to 12 years for premature parturient females and 10 to 15 years for full-term parturient females. The numbers in parentheses are standard deviations.

| Tissue | *N* | Mean age (years) | Fat (%) | | DDT (total) | | PCB's | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | Range | $\bar{X}$ (ppm) | Range (ppm) | $\bar{X}$ (ppm) | Range (ppm) |
| | | | | *Premature* | | | | |
| Blubber (female) | 6 | 8 | 84 | 77–87 | 824.4 (167.8) | 626–1039 | 112.4 (24.4) | 85–145 |
| Liver (female) | 3 | 8 | 1.7 | 1.5–2.2 | 25.24 (4.1) | 22–30 | 5.74 (3.45) | 3.4–9.7 |
| Brain (pup) | 6 | | 3.3 | 3.2–3.4 | 2.38 (1.27) | 0.82–4.15 | 0.45 (0.32) | 0.23–1.05 |
| | | | | *Full-term* | | | | |
| Blubber (female) | 4 | 12 | 85 | 84–88 | 103.2 (69.7) | 51–203 | 17.1 (6.1) | 12–25 |
| Liver (female) | 4 | 12 | 4.4 | 2.9–5.5 | 6.67 (5.1) | 2.4–13.6 | 1.32 (0.76) | 0.52–2.16 |
| Brain (pup) | 4 | | 4.8 | 4.6–5.1 | 1.20 (1.10) | 0.36–2.82 | 0.19 (0.05) | 0.12–0.23 |

ture partus females except one. The liver of this animal showed a few midzonal foci of mononuclear and polymorphonuclear cell infiltrates. These abnormal findings apparently are not similar to histological changes in rats fed organochlorine pesticide (*7*) or rodents fed PCB compounds (*8*). Serological analysis revealed no antibody titers to *Leptospira* species or *Brucella abortus* in the six cows of the premature parturient group. Bacteriological analysis of the uteri of this group yielded isolation of *Escherichia coli* from two of the six.

Liver mercury residues (wet weight) in the three premature parturient females ranged from 38 to 64 parts per million (ppm) and in their pups ranged from 0.4 to 1.8 ppm. None of the tissues from full-term animals were analyzed for mercury.

The difference between the concentrations of organochlorine pesticide and PCB residues in the premature and full-term groups indicates a possible cause and effect relationship between high levels of these pollutants and early termination of pregnancy in California sea lions. High concentrations of organochlorine residues are implicated in reproductive failure in ranch mink fed fish from Lake Michigan (*9*). Experimental feeding of PCB compounds has been shown to cause reproductive failure and death in adult ranch mink (*10*). There is also experimental evidence that *p,p'*-DDT induces premature parturition in rabbits (*11*). Some organochlorine insecticides and PCB compounds induce hepatic microsomal enzyme activity resulting in increased metabolism of progesterone and estradiol in pigeon livers (*12*). Similar enzyme induction has been demonstrated in the rat (*7*). Definitive knowledge of the combined toxic or synergistic effects of organochlorine pesticides, PCB's, and mercury is lacking. LeBoeuf and Bonnell (*13*) reported high residue levels from three "apparently healthy" female California sea lions collected in September 1970 at San Miguel Island. Apparent good health of the female does not exclude a history of premature parturition. Since they did not report the reproductive histories of those animals, it is impossible to relate their findings to the premature parturition phenomena.

The exact relationship of pollutants to premature births and the mechanisms involved can only be elucidated through laboratory experiments. Such experiments with California sea lions have not been possible because of the cost of maintaining large breeding colonies of sea lions in captivity. However, considering the magnitude of the differences in residue levels found in the two groups sampled from the wild population, we feel that the possible cause and effect relationship cannot be ignored.

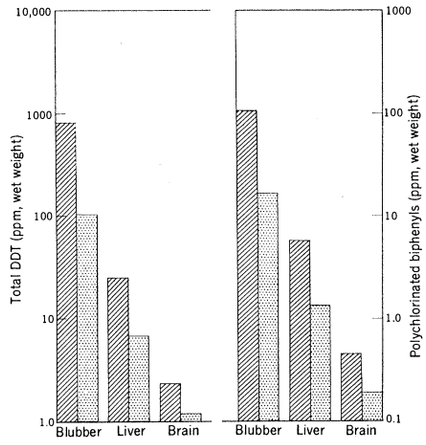


Fig. 1. Histogram of mean concentrations of total DDT and PCB's in tissues of California sea lions delivering prematurely (cross-hatching) and at full term (stippling).

A possible explanation for the difference in pollutant residue levels in the tissues from the two groups of sea lions is utilization of different feeding areas. It has long been known that after the breeding season male California sea lions move northward. It is believed by some that many females of the Channel Islands population move south and winter in waters off southern Baja California (*14*), while a portion remain in the general area of the Channel Islands.

Organochlorine residues in a marine crab (*15*), marine fish (*16*), and the brown pelican (*17*) are higher around Los Angeles, California, than in Baja California or in northern California. Thus, female sea lions wintering in the southern areas would be feeding on fish having smaller amounts of organochlorine pesticides and would therefore assimilate smaller quantities of the pesticides than females wintering in the Channel Islands area.

The mean age of premature parturient females is less than that of full-term females (Table 1), but the age ranges of the groups overlap. Because of the small sample sizes and the overlap in age ranges it is not possible at this time to relate the observed difference in organochlorine residues or the premature parturition phenomena to age.

We think it is unlikely that the observed difference in organochlorine residue concentrations could be accounted for by the premature parturient females being collected 2 months before the full-term females. Five sea lion females collected randomly from the San Miguel Island population in July 1969 had total DDT metabolite residues in the blubber ranging from 17 to 988 ppm (*18*), which indicates that some females can be expected to have high residues at any time of year and that a profile of the population probably ranges from animals with low to those with very high concentrations of organochlorine residues. Unfortunately, nothing is known about the fat dynamics in gestating female sea lions or about changes in organochlorine residue storage dependent on hormone cycles. Net mobilization of fat to meet increasing energy demands would be expected to increase residue levels in the blubber, yet we found that the full-term females had lower organochlorine residue concentrations.

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## Cell-Mediated Immune Responses in vitro: Interaction of Thymus-Derived Cells during Cytotoxic Allograft Responses in vitro

Abstract. *A mouse in vitro allograft system was used to check for synergism among thymus-derived cells during cell-mediated immune responses. Synergistic interactions occurred between thymus and thymus-derived lymphocytes. The majority of precursors of cytotoxic effector cells were peripheral thymus-derived cells; thymocytes acted mainly as amplifier cells.*

Both murine thymocytes and thymus-derived (T) lymphocytes have been shown to be capable of mounting cytotoxic allograft responses in vivo (*1*) and in vitro (*2*), apparently without requiring collaboration with bone marrow (B)–derived lymphocytes. When compared on a cell to cell basis, thymocytes were found to be poor precursor cells of cytotoxic lymphocytes (CL) in vitro in contrast to recirculating T cells, splenic T cells being intermediate (*2*). These findings suggested that there exists a heterogeneity within thymus-processed lymphocytes in their capacity to differentiate in vitro into cytotoxic effector cells. Indeed evidence for the existence of functional T cell subpopulations has appeared (*3, 4*). For example, Cantor and Asofsky (*4*) proposed a model for the generation of thymus-derived effector cells mediating graft-versus-host reactions, in which two distinct types of T cells appear to be involved: one acting as an amplifier cell and the other type functioning as the precursor of the effector cell. I have now tested the concept of T-T cell interactions during cell-mediating immune responses in an in vitro allograft system.

About $15 \times 10^6$ viable dissociated CBA/H/Wehi (H-$2^k$) or AKR/J (H-$2^k$) mouse thymocytes or inguinal lymph node (LN) cells, or a mixture of both, were cultured together with $2 \times 10^6$ mitomycin C–treated BALB/c spleen cells (H-$2^d$) for 6 days in modified Eagle's medium containing 5 percent fetal calf serum in the Marbrook-Diener culture system (*2, 5*). Cells from three cultures per group were pooled, washed twice, and assayed for in vitro generated cytotoxic activity in a modification (*2*) of the $^{51}Cr$ release assay described by Brunner *et al.* (*6*). Cytotoxic activity generated in vitro was compared on a culture basis (*7*). Thus, the number of lymphocytes at the initiation of the culture was kept identical in the various groups of cultures, and the cytotoxic activity generated was compared relative to that obtained with LN cells as precursor cells of cytotoxic lymphocytes. In actual fact, cytotoxic lymphocytes harvested from different groups were assayed for cytotoxic activity at the cell concentration which resulted (in the case of LN derived cytotoxic lymphocytes) in a ratio of cytotoxic lymphocytes to target cells of 12 to 1, 2 to 1, and 0.3 to 1. The DBA/2 derived mastocytoma cell line P815 (*8*), cultured in vitro and of identical H-2 specificity as BALB/c spleen cells used for immunization, was used as target cells in the $^{51}Cr$ release assay. AKR antiserum to CBA $\theta$ antigen was prepared according to Raff (*9*) and used as described (*7*). The antiserum killed 80 percent of CBA thoracic duct lymphocytes in the presence of agarose-absorbed guinea pig serum, and the activity against $\theta$ could be absorbed by brain from CBA mice. Mitomycin C treatment was administered at a final concentration of 40 $\mu$g/ml for 30 minutes (*2*). Irradiation (900 rads) of dispersed lymphocytes was performed as described by Miller and Sprent (*10*).

As given in Table 1, CBA LN cells (an example of a lymphocyte population containing 80 percent of peripheral T cells) represented a good precursor cell population for cytotoxic lymphocytes. Thus, after a 6-day culture period in the presence of cell-bound alloantigen ($2 \times 10^6$ mitomycin C–treated BALB/c spleen cells), cytotoxic activity was generated which lysed 80 percent of the $^{51}Cr$-labeled P815 target cells at a ratio of cytotoxic lymphocytes to target cells of 2 to 1 within 200 minutes. That the reacting cells were T lymphocytes was suggested by the fact that treatment of LN cells before culture with AKR antiserum to $\theta$ antigen, plus complement, abolished the capacity of the cells to mount a cytotoxic

Table 1. Synergistic effect of a mixture of thymocytes plus syngeneic lymph node cells in cytotoxic allograft responses in vitro.

| Test system | Lysis (%)* of $^{51}Cr$-labeled P815 target cells (H-$2^d$) at a CL to target cells ratio of: | | |
|---|---|---|---|
| | 12 : 1 | 2 : 1 | 0.3 : 1 |
| $15 \times 10^6$ CBA LN | 100 | 80 | 40 |
| $15 \times 10^6$ CBA LN, treated with antiserum to $\theta$ | 13 | 12 | 12 |
| $15 \times 10^6$ CBA thymocytes | 30 | 14 | 12 |
| $1.5 \times 10^6$ CBA LN plus $14 \times 10^6$ CBA irradiated thymocytes | 25 | 13 | 12 |
| $1.5 \times 10^6$ CBA LN plus $14 \times 10^6$ CBA thymocytes | 100 | 91 | 52 |
| $1.5 \times 10^6$ (CBA LN), treated with antiserum to $\theta$ plus $14 \times 10^6$ CBA thymocytes | 25 | 14 | 12 |
| $1.5 \times 10^6$ (CBA $\times$ BALB/c) $F_1$ LN plus $14 \times 10^6$ CBA thymocytes | 23 | 13 | 12 |
| $1.5 \times 10^6$ CBA LN plus $14 \times 10^6$ (CBA $\times$ BALB/c) $F_1$ thymocytes | 38 | 27 | 13 |
| $1.5 \times 10^6$ (CBA LN) mitomycin C–treated plus $14 \times 10^6$ CBA thymocytes | 24 | 12 | 12 |

* Responder cells (H-$2^k$) were cultured together with $2 \times 10^6$ mitomycin C–treated allogeneic BALB/c (H-$2^d$) spleen cells. Cytotoxic activity generated in vitro was assayed in a $^{51}Cr$ test for 200 minutes. Cytotoxicity obtained was compared on a culture basis relative to the cytotoxicity generated by CBA LN cell derived cytotoxic lymphocytes. Background lysis of P815 target cells (in the presence of normal lymphocytes) was $12 \pm 1.3$ percent. Similar results were obtained in 12 independent experiments.