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Project Summary

Sensitive Biochemical and Behavioral Indicators of Trace Substance Exposure: Part 1. Cerium

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The overall objective of this project was to investigate potential toxic effects of acute and chronic exposure to Ce with the mouse as a model mammalian system. In the adult mouse, the tissue/organ distribution of Ce was determined at various times after exposure to a single dose or repeated (multiple) doses of CeC13. In addition, the effects of Ce exposure on selected behavioral parameters were also examined along with open-field and exploratory behaviors, passive and active avoidance learning, and an aspect of social behavior. Statistical correlations between tissue/organ Ce levels and various behavioral measures were also examined in the adult studies to gain insight into the basis of the relationships between the two sets of data. Another major focus of this research examined the effects of Ce. on the gravid female mouse and on the embryonic, fetal and postnatal development of the offspring of Ce exposed dams. Cerium tissue/organ distribution and various maternal and offspring developmental behaviors were investigated.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project which is fully documented in a separate report of the same title (see Project Report ordering information at back).

Discussion

Subcutaneous administration of 136 mg Ce/kg (LD_5) was found to induce fatty infiltration of the liver. However, this effect was transient: the fatty infiltration regressed with time while the liver Ce levels remained elevated. The nature of this phenomenon was probed in studies of the subcellular distribution of Ce in the liver as a function of time.

Lethality parameters were established for Ce (citrate) administered intragastrically (i.g.) and subcutaneously (s.c.). The i.g. route was selected for its environmental relevance and the s.c. route for better dosage control and for its relation to the transdermal route.

For males, the LD_{50}, LD_{25} and LD_5 (with 95% confidence intervals) levels were: 1291 (1198-1449), 1163 (1017-1250) and 1000 (743-1104) mg Ce/kg, respectively, for the i.g. route of administration and 205 (181-241), 173 (140-195) and 136 (86-160) mg Ce/kg, respectively, for the s.c. route.

Nongravid and gravid (day 12 of gestation) females received 68 to 318 mg Ce/kg. Day seven viability and

Lethal dose levels (calculated by probit analysis) for the s.c. route of administration are summarized in Tables 1 and 2, respectively.

The tissue distribution of Ce was investigated in adult males and females, in the developing fetus and in offspring of Ce exposed dams. Male mice were exposed either to a single, acute dose or to a multiple dose sequence.

For single dose exposure, all tissues/organs exhibited significant main effects of time and/or route. The liver, kidney, lung, muscle, cerebrum and brain stem also showed an effect of dose but only within the s.c. route. Many tissues exhibited a time-by-route interaction but only lung exhibited a dose-by-time interaction and-only-via the s.c. route. The strongest effect by far was that of route. This was anticipated and largely reflects the poor uptake of Ce via the i.g. route as well as quantitative differences in the actual doses administered via each route. The time effects were due to the time dependent decrease in tissue Ce (except in the spleen where Ce peaks between one and three days post administration). The significant dose effects via the s.c. route were due to higher tissue/organ Ce levels in animals receiving the higher Ce dose. The absence of such effects in some tissues may be accounted for by the high variability encountered at the doses employed. The time-by-route and time-by-dose interactions within the s.c. route may be attributed to route/dose pharmacokinetic differences.

The tissue/organ Ce levels for the multiple dose exposure are presented in Table 3. The multivariate ANOVA showed that, across all tissues, there was a significant difference in Ce concentration across exposures ($F = 2.27$, d.f. = 126/510.92, p 0.001). The univariate F-ratios showed that this was also true (p 0.05) for all individual tissues except blood, cerebellum and brain stem. For the most part, the effect was one of increasing Ce tissue/organ levels with the increasing number of exposures. Muscle levels were highly variable and may reflect some direct absorption of Ce via migration from the injection sites.

Distribution of Ce in the tissue of mother, fetus, and offspring was also determined. Cerium levels were highest in all maternal tissues one to two days after Ce administration and then declined through the remainder of the gestation period. The highest Ce level observed was in liver (333 ± 23 ppm) two day post administration. The Ce levels in maternal tissues were of the order: liver > spleen > lung > kidney. Maternal brain Ce levels were low (less than 0.3 ppm) at all times of observation. Cerium levels in the developing organism were highest one day post administration (5.71 ± 1.22 ppm) and then declined six fold over the succeeding four days. Cerium was below the level of detectability (limit of detection < 0.05 ppm) in the individual tissues selected for examination of offspring of all treatment groups from parturition up to 21 days *post partum*. Neonates had measurable whole body Ce levels up to three days post maternal Ce administration. However, Ce was undetectable in fetal blood, liver, kidney, cerebrum and cerebellum even though mean whole body content measured 0.12 ppm $\pm$ 0.02 ppm.

Effects of exposure to acute and repeated doses of Ce, via the i.g. or s.c. routes, on selected behavioral parameters were also investigated. These data were correlated with those of the tissue distribution study in an attempt to gain insight into the mode of toxic action of Ce. These studies included both single (acute) and multiple dose exposures. Acute exposure studies included measures of open-field behavior, exploratory behavior, wheel usage, passive and active avoidance, and social behavior. Significant effects were found for all measures.

The effects of repeated exposure to Ce on open-field and exploratory behavior were studied and correlated with tissue levels of Ce. A significant multivariate effect was found for number of exposures, and significant univariate effects were found for rearings and exploration.

Developmental studies included neonatal, maternal, and adult offspring

Table 1. *Effect of Increasing Ce Dose[a] on Seven Day Viability or Gravid[b] and Nongravid Animals*

	Seven Day Viability	
Dose Level	Nongravid	Gravid
68	—	10/10
86	—	10/10
104	—	9/10
122	10/10[c]	8/10
140	10/10	8/10
157	8/10	5/10
175	7/10	5/10
192	4/10	3/10
210	6/10	1/10
228	6/10	1/10
246	2/10	0/10
264	1/10	0/10
282	1/10	—
300	0/10	—
318	0/10	—

[a] *The Ce dose administered was based on maternal weight at time of injection.*

[b] *All mice were injected on day 12 of gestation.*

[c] *Numerator = number surviving; denominator = total N.*

Table 2. *Ce Lethal Dose Levels as Determined by Linear Regression Analysis of Seven Day Viability*

	Nongravid		Gravid[d]	
Lethality[a]	Dose[b]	Range[c]	Dose	Range
LD_1	117	71.6-142	80.2	43.6-103
LD_5	138	95.8-161	94.4	55.8-119
LD_{11}	151	112-172	108	73.1-129
LD_{25}	174	143-193	131	100-153
LD_{40}	193	168-212	150	127-172
LD_{50}	205	183-226	166	147-189
LD_{60}	218	198-244	179	160-204
LD_{75}	241	220-283	199	181-237
LD_{90}	280	249-360	226	198-280
LD_{95}	305	266-419	254	218-361
LD_{99}	360	300-559	301	251-497

[a] *Lethality levels are expressed as the percent mortality expected within seven days after Ce administration.*

[b] *Ce dose, in ppm administered on the basis of maternal weight, calculated to produce the corresponding lethality.*

[c] *The $p<0.05$ probability range of the corresponding dose.*

[d] *All mice were injected on day 12 of gestation.*

Table 3. *Tissue/Organ Ce Concentrations in µg Ce/g (Wet Weight) Tissue (PPM) - Repeated Exposure*

Cumulative dose (mg Ce/kg) ppm[a] *N (subjects per cell)*

Time (days) after first exposure

			Blood[b]		Liver		Kidney		Spleen		Pancreas		Lung	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
20	3	10	0.04	0.03	28.80	7.14	1.09	0.38	27.34	10.25	0.19	0.09	2.59	0.45
40	6	10	0.03	0.03	47.53	3.32	3.18	5.17	38.21	15.36	0.24	0.07	3.71	1.08
60	9	10	0.04	0.04	73.36	16.72	3.23	1.17	83.65	43.43	0.59	0.56	11.32	12.46
80	12	10	0.05	0.06	81.93	16.50	3.63	0.90	94.54	44.76	0.60	0.21	9.07	2.86
100	15	10	0.06	0.05	111.02	19.76	5.08	1.28	133.74	33.61	0.94	0.78	9.07	1.12
120	18	8	0.04	0.02	130.59	29.46	6.23	1.13	175.98	64.62	0.86	0.27	12.42	3.58
140	21	7	0.03	0.03	118.38	11.60	5.50	0.95	160.55	35.99	0.82	0.42	13.19	1.65
160	24	9	0.04	0.03	147.31	24.97	6.18	2.63	195.25	46.02	1.10	0.48	14.67	2.85
180	27	7	0.03	0.03	151.61	19.24	6.23	2.57	227.88	96.71	0.98	0.18	15.95	5.10
200	30	7	0.04	0.02	155.27	37.32	6.55	2.15	228.50	118.61	1.08	0.37	16.27	5.01
200	69	9	0.09	0.23	157.06	57.51	7.56	3.10	315.83	217.35	1.16	1.47	14.24	4.83
200	125	6	0.18	0.16	152.78	44.73	6.01	1.57	341.32	124.05	1.84	1.20	13.49	2.38

			Stomach		Duodenum		Testes		Skeletal Muscle	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
20	3	10	0.68	2.27	0.78	0.37	0.37	0.12	0.11	0.07
40	6	10	0.87	0.37	0.96	0.41	0.54	0.24	32.04	44.46
60	9	10	1.67	0.61	2.11	0.75	0.82	0.17	24.53	26.53
80	12	10	2.21	0.55	2.40	1.22	1.14	0.75	23.12	28.25
100	15	10	2.92	1.35	3.59	1.98	1.38	0.35	89.54	114.56
120	18	8	3.29	1.28	4.31	1.86	1.72	0.46	32.62	26.89
140	21	7	2.99	1.56	2.87	0.43	1.51	0.35	35.91	56.17
160	24	9	4.05	1.46	3.80	1.18	1.84	0.25	47.71	42.37
180	27	7	3.69	1.60	4.49	1.82	1.83	0.33	30.18	29.18
200	30	7	4.65	2.27	3.59	1.53	1.84	0.54	26.90	17.90
200	69	9	4.51	2.05	3.77	3.93	1.85	0.68	37.80	44.57
200	125	6	4.03	2.91	2.63	1.11	2.09	0.67	54.25	50.94

			Bone		Cerebrum[b]		Cerebellum[b]		Brain Stem[b]	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
20	3	10	3.71	1.14	0.09	0.05	0.2	0.2	0.09	0.07
40	6	10	6.69	5.41	0.05	0.05	0.2	0.2	0.06	0.06
60	9	10	12.70	3.74	0.13	0.11	0.3	0.3	0.08	0.08
80	12	10	16.48	10.69	0.07	0.03	0.2	0.2	0.10	0.08
100	15	10	22.02	12.98	0.08	0.03	0.2	0.2	0.12	0.07
120	18	8	26.62	11.70	0.16	0.08	0.3	0.3	0.16	0.10
140	21	7	15.74	5.82	0.11	0.06	0.3	0.3	0.14	0.08
160	24	9	21.02	9.81	0.13	0.06	0.2	0.1	0.12	0.10
180	27	7	34.01	29.18	0.13	0.05	0.3	0.2	0.16	0.07
200	30	7	27.60	13.38	0.12	0.06	0.3	0.2	0.13	0.12
200	69	9	30.91	9.04	0.13	0.11	0.2	0.3	0.19	0.25
200	125	6	39.17	9.01	0.77	0.65	1.3	1.2	1.13	0.47

[a] *Each dose (s.c.) contained 20 mg Ce/kg body weight as the $CeCl_3$: sodium citrate, 1:3 complex, pH 7.4. Animals were sacrificed three days after their last injection except for the last two groups which were sacrificed 42 and 98 days after their last injection.*

[b] Concentrations near detection limit and should be regarded as upper limits rather than accurate values.

observations. Significant effects were found in all cases.

To obtain information on the relationship of the subcellular distribution of Ce to the genesis and rapid disappearance of Ce induced fatty infiltration of the liver, the subcellular distribution of Ce was investigated.

One day post administration, the livers of the Ce exposed animals exhibited signs of fatty infiltration. They were mottled and light in color and, following centrifugation of the homogenates to obtain the nuclear pellet, the supernatants displayed evidence of lipid levels considerably higher than those found in control liver homogenates.

A representative subcellular distribution of Ce in the liver (obtained utilizing density gradient procedure B described above) at one, three and seven days post administration is presented in Table 4. The ANOVA of the distribution, as a function of time, is presented in Table 5. Two animals in the one day group had extraordinarily high percentages of Ce in their nuclear fractions and low percentages in their pellets. (Other than evoking individual variability, there is, at present, no explanation for this finding). Thus, the means and standard deviations for the nuclear and "Pellet" fractions are presented, both including and excluding these animals.

The highest percentages of recovered Ce was found in the "Pellet." This fraction is comprised of the materials having a density greater than that of the nuclear fraction. The percentage of Ce in the "Pellet" increased from 45 to 53 from one to seven days post exposure. The nuclear fraction contained the second highest percentage of Ce. However, in contrast to the "Pellet" fraction, the percentage of Ce in this fraction decreased from 28 to 14 from one to seven days post administration. The mitochondria contained the third highest percentage of Ce which remained relatively invarient throughout the period of observation. As in the "Pellet," the percentages of Ce in both the microsomes and the cytosol increased across time, while the peroxisome content decreased to a constant level at three days. The differences in Ce content across time were significant for all fractions except the mitochondrial.

Conclusions

The Tissue/Organ Distribution and Alterations in Open-Field and Exploratory

Table 4. *Liver Subcellular Ce Distribution at One, Three and Seven Days Post Administration (Percent of Ce Recovered)*

	Time post administration (days)					
	1		3		7	
Fraction	Mean	SD	Mean	SD	Mean	SD
Cytosol	2.34	0.39	4.40	0.73	7.17	0.53
Microsomes	7.32	1.29	12.49	0.88	13.92	1.52
Mitochondria	10.33	1.80	10.09	2.21	8.28	1.30
Peroxisomes	6.37	1.40	3.64	0.73	3.18	0.73
Nuclei	36.70 (28.16)[a]	14.31 (4.22)[a]	17.49	4.95	14.14	2.01
"Pellet"[b]	37.47 (45.73)[a]	13.32 (4.05)[a]	51.88	2.89	53.29	1.60
% of Ce Recovered	96.34	1.73	90.64	2.82	88.49	3.55

[a]*Values obtained omitting two animals with extraordinarily high nuclei and low pellet values.*

[b]*This term denotes all materials with a density greater than that of the nuclear fraction.*

Behavior Following Accute Exposure to Ce. Between four hours and seven days post administration, Ce administered via the i.g. route at a level as high as the LD_{25}, had no significant effect on open-field (ambulations and rearings) or "hole-in-board" exploratory behaviors of the adult mouse. Apparently, this was due to the fact that little Ce is absorbed from the gut. Despite low uptake, the doses employed were toxic to the mouse. Gastritis and enteritis were demonstrated histologically in these animals. However, it is not known if Ce was directly responsible for such lesions. It is possible that osmotic phenomena may have been involved in view of the high solute concentrations employed.

A different situation prevailed when Ce was administered via the s.c. route. At short times post administration, open-field and exploratory behaviors were significantly depressed, systemic distribution of Ce was substantial and behavioral alterations were correlated with tissue/organ Ce levels.

An inverse relationship between behavior, brain and lung Ce levels was found. Of all tissues studied, the brain correlated most strongly with behavior. This is of particular interest in that the same treatment groups which had exhibited depressed open-field behavior (which occurred at four hour and one day post s.c. administration of Ce at the LD_{25} level and at four hour post administration of the LD_5 dose) also exhibited detectable brain Ce levels. In contrast, the brain Ce levels of all other groups were essentially at or below the detection limit. Moreover, there are numerous reports in the literature of lanthanum interfering with "calcium sites" on nerve cells. Cerium, the next element in the lanthanide series, may have a similar effect which, in this study, was expressed as depressed open-field and exploratory behavior.

After the brain regions, the strongest behavioral correlation involved the lung. The lung accumulated substantial quantities of Ce. However, histological examination revealed no abnormalities attributable to Ce. Thus, the relationship

Table 5. *Analysis of Variance of Liver Subcellular Ce Distribution*

Variable	Time Effect
Multivariate d.f.	12/20
F-ratio	13.67[a]
Univariate d.f.	2/15
F-ratios	
Cytosol	118.72[a]
Microsomes	45.66[a]
Mitochondria	2.01
Peroxisomes	17.75[a]
Nuclei	11.08[a] (16.26)[a]
Pellet	7.23[b] (9.22)[bd]
% Ce Recovered[c]	11.22[a]

[a]$P < 0.001$.

[b]$P < 0.01$.

[c]*Not included in the multivariate F-ratio.*

[d]*Values obtained omitting 2 animals in the 1 day group with extraordinarily high nuclei and low pellet values.*

between lung Ce levels and behavior remains unclear. These data suggest that inhalation of Ce may be a considerably greater hazard than ingestion.

The spleen is of interest in that it contained the highest Ce concentration of any tissue examined. Its Ce level was the only one positively correlated with behavior. The spleen may have acquired its Ce load from damaged erythrocytes and leucocytes. The high Ce concentration in the spleen and the positive correlation with behavior suggest that sequestration of Ce by the spleen may function to spare more sensitive potential target tissues, such as the brain, from perturbation. Indeed, the small negative correlations which were observed between Ce levels in the spleen and brain regions are consistent with such a "protective" function.

It is possible, of course, that the hypoactivity observed in the open-field behavioral measures of animals receiving Ce via the s.c. route was mediated by a secondary effect of Ce, namely, that it made the animals physically ill and lethargic. However, it should be noted that no behavioral effects were observed in animals receiving Ce via the i.g. route, even though histopathological analysis provided evidence of acute gastritis and enteritis with a duration of at least seven days post administration.

If the animals were hypoactive simply because they were physically ill, it would be anticipated that the i.g. dosed animals would certainly have been physically ill at least through day seven post administration. The s.c. dosed animals which showed no evidence of gastritis or enteritis exhibited significant open-field behavioral alterations which were strongly correlated with brain Ce levels. Furthermore, s.c. dosed animals also exhibited alterations in exploratory behavior. The exploratory task employed was substantially less dependent on the gross activity level of the animals and, therefore, suggests that the effects of Ce were not limited to a simple decrease in the general activity level.

In summary, Ce administered via the s.c. route achieves general systemic distribution and depressed open-field and exploratory behavior up to 24 hours post administration, possibly by interaction with the CNS.

High Ce doses administered i.g., while toxic, did not effect either ambulations or rearings in the open-field nor exploratory behavior. Probably, this was due to the poor uptake of Ce from the gut which consequently limited systemic distribution. Peroral exposure to Ce would appear to present little danger of acute toxicity, since the doses used in these studies were considerably greater than any anticipated to occur from environmental exposure.

If Ce compounds are incorporated into fuels, inhalation will constitute another route of environmental exposure. A much higher percentage of Ce appears to be retained following inhalation than ingestion. However, even via s.c. administration, the lung accumulates Ce and lung Ce levels are inversely correlated with open-field activity. These findings suggest that inhalation of Ce may be a considerably greater acute hazard than ingestion and must be investigated before Ce (compounds) can be considered "safe" for widespread environmental dispersion

Activity Wheel Study The results of this study indicate that a single dose of Ce at the s.c. LD_5 level can significantly depress general activity and that this effect is persistent. This relative depression was maintained, along with a monotonic increase in performance, for all groups (LD_5, LD_{25} and controls) over time. The most depressed scores for the Ce exposed groups occurred during the 24 hour period following Ce administration. It is difficult to interpret this effect, since the animals did not exhibit any obvious signs of illness or incapacitation. It appears that a single exposure to Ce, at relatively high levels (LD_5, LD_{25}), has a substantial effect on total (24 hour) activity level. Perhaps total activity, as assessed by the activity wheel, is a more sensitive index of subtle effects induced by Ce exposure than is the short time sample obtained with such measures as the open-field.

Passive Avoidance Compared to sodium citrate, acute Ce citrate administration was associated with an increase in latency in both trails of the passive avoidance learning task. This was probably another manifestation of the depressed activity observed in the acute open-field and activity wheel studies. Relative to the citrate controls, learning of the passive avoidance task was not adversely effected by Ce administration.

Active Avoidance Behavior Single doses of Ce at the LD_5 and LD_{25} levels had no effect on two-way active avoidance learning by the mouse. However,consistent with the results of the open-field, activity wheel, and passive avoidance studies (see above), Ce at the s.c. LD_{25} level depressed activity. The depressed activity did not influence the learning of the task.

Social Behavior As in the open-field, activity wheel, passive avoidance and active avoidance studies, Ce (at the LD_5 and LD_{25} levels) depressed gross activity. Thus, the control (citrate) animals were significantly more active than either group of Ce exposed animals (which were not significantly different). Although mobility was depressed, Ce had no dramatic effect on the social behaviors that were investigated. However, multivariate ANOVA of the data revealed a dosage by day of observation interaction. This interaction is difficult to assess. Taken at face value, it would appear that Ce, at high dose levels (LD_{25}), does affect a measure of social behavior (e.g., distance maintained between pairs of animals), but the effect has a long latency period and does not appear until 3 days post exposure The absence of more consistent patterns of effects on social behavior indicates that further study is required before confident conclusions can be generated.

The Tissue/Organ Distribution and Alterations in Open-Field and Exploratory Behavior Following Exposure to Repeated Doses of Ce Exposure to repetitive s.c. doses of Ce citrate at the LD_1 level resulted in the accumulation of Ce mainly in the spleen, liver and skeleton. No decrease in Ce levels was observed in these tissues up to 98 days after the last dose was administered. Cerium levels in the liver, kidneys, pancreas and testes and, possibly, in the stomach and duodenum appeared to plateau following the fifth or sixth dose However, spleen and bone continued to accumulate Ce even after exposure was ended. Blood and brain levels were very low exhibiting counting rates (^{141}Ce) near background. It is of interest to note that the blood Ce levels remained low throughout the course of the experiment. Similar results were found in the acute s.c. study (see above). Cerium obviously is cleared very efficiently from the blood Judging from their Ce concentrations across time, the spleen, liver and bone appear to be involved in the clearance mechanism.

Although Ce is rapidly cleared from the blood, it is not rapidly excreted and toxic effects could result from long-term retention unless Ce is sequestered in some non-toxic form.

Exposure to repetitive s.c. doses of Ce significantly depressed ambulations in

the open-field and marginally depressed "hole-in-board" explorations across all subjects. However, these behavioral measures did not correlate with tissue/organ Ce levels which indicate that the depressive effect of Ce is not a one step process depending directly on Ce levels in some tissue(s). Rather, it probably involves a chain of events culminating in depressed activity. Such a process is not uncommon in biological systems.

In the acute study described previously, highly significant depressions of ambulations, rearings and explorations at four hours and one day post s.c. administration occurred. The doses employed in that study were much higher (136 and 173 mg Ce/kg) than the individual doses (20 mg Ce/kg) used in the repeated dose study. This could account for the differential behavioral results. Detectable Ce levels were found in the brain coincident with depressed behavior in the acute study, whereas no detectable brain Ce levels were found in the repeated dose study. It may be that the depression of ambulations and exploratory behavior observed in the repeated dose study was due to small amounts of Ce in the central nervous system. However, the magnitude of this relationship was too small to detect statistically given the sample size and the sensitivity of the Ce assay.

Both the acute and repeated dose studies demonstrated that exposure to Ce can induce behavioral changes in the mouse. It seems reasonable to conclude that widespread environmental dispersion of Ce could be potentially hazardous considering these findings and the long biological residence time of Ce. However, much more information must be accumulated before the risks of environmental Ce dispersion can be accurately assessed.

Neonatal and Adult Offspring Studies Maternal administration of Ce on Day 12 of gestation affected the development of certain offspring behaviors. Conversely, offspring of mothers receiving Ce on Day seven of gestation did not differ from control offspring in the neonate and adult behavioral measures investigated. Whether the differential effects of day of exposure were due to differences in the intrinsic sensitivity of the developing organism at these stages of gestation or to placental permeability factors were not examined.

The most consistent effect of Ce was on offspring weight. Neonatal weight was reduced in offspring exposed to Ce *in utero* via maternal administration and in the offspring reared by mothers who received Ce on Day two *post partum:* during lactation/suckling. The significant prenatal X postnatal interaction indicates that the treatment the foster mother receives can modify the rate of weight gain of the offspring she rears. Thus, exposure of pregnant females to a single dose of Ce at the s.c. LD_1 level had a relatively long-lived effect on the mother which was reflected postnatally in a decreased rate of offspring growth. This effect may be direct, through residual Ce in the milk of mothers treated during gestation, or indirect, through effects on maternal behavior manifested, for example, as neglect of offspring, and reflected in such factors as ineffective suckling or lack of grooming.

The latter possibility appears reasonable since laboratory studies revealed that Ce is not transmitted to offspring via the milk of mothers exposed during gestation to the dose levels of Ce employed. However, the reduced weights observed in offspring exposed to Ce prenatally may indicate for example, that the developing fetuses received inadequate nutrition because of covert maternal intoxication resulting in impaired transport of nutrients across the placenta, Ce induced metabolic dysfunction in the offspring that resulted in impaired ability to utilize nutrients, the production of milk of inferior nutritional quality or, perhaps, all, or combinations, of these possibilities.

Maternal Behavior The offspring retrieval data indicate that pups exposed to Ce *in utero* are retrieved in preference to control pups. Unfortunately, activity was not tested on Day three *post partum*, the day retrieval was tested. Conceivably, Ce pups were preferentially retrieved because they were less active and of lighter weight.

Liver Subcellular Distribution of Ce The highest percentages of Ce were found in the pellet (about 50%) and the nuclei (28% decreasing to 14%). About half of the Ce found in the nuclei of cells examined one day post exposure appeared to have moved into the pellet, microsomal and cytosolic fractions of cells by three to seven days post exposure. While no data are available on the mechanism of this transfer, Ce may be transported out of the nuclei bound to mRNA produced in conjunction with regenerative processes in the liver. Cerium may exist in the pellet in the form of insoluble phosphates and hydroxides. Insoluble forms of Ce also may be trapped within organelles or bound to macromolecules (proteins, RNA or DNA) that are normally associated with them.

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The complete report, entitled "Sensitive Biochemical and Behavioral Indicators of Trace Substance Exposure: Part 1. Cerium," (Order No. PB 81-150 765; Cost: $8.00, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
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The EPA Project Officer can be contacted at:

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