THE TOXICOLOGY AND METABOLISM OF
NICKEL COMPOUNDS

Progress Report

for Period December 1, 1975 - November 30, 1976

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August 15, 1976

Prepared for

THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

UNDER CONTRACT NO. E(11-1)-3140

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Abstract of Progress Report

Attachment to U.S. ERDA Research Document No. COO-3140-35

"The Toxicology and Metabolism of Nickel Compounds"

The toxicology and metabolism of nickel compounds (NiCl_2 , Ni_3S_2 , NiS , Ni powder and $\text{Ni}(\text{CO})_4$) have been investigated in rats and hamsters. The new knowledge has included:

(1) Demonstration that triethylenetetramine (TETA) and d-penicillamine are more effective than other chelating agents (Na-diethyldithiocarbamate, CaNa_2 -versenate, diglycylhistidine-N-methylamide and α -lipoic acid) as antidotes for acute $\text{Ni}(\text{II})$ -toxicity in rats.

(2) Demonstration that the antidotal efficacy of triethylenetetramine (TETA) in acute $\text{Ni}(\text{II})$ -toxicity is mediated by rapid reduction of the plasma concentration of $\text{Ni}(\text{II})$, consistent with renal clearance of the TETA- Ni complex at a rate more than twenty times greater than the renal clearance of non-chelated $\text{Ni}(\text{II})$.

(3) Demonstration that Fischer rats are more susceptible than other rat strains (Wistar-Lewis, Long-Evans and NIH-Black) to induction of erythrocytosis after an intrarenal injection of Ni_3S_2 , and elucidation of the serial pathologic changes that occur in rats after an intrarenal injection of Ni_3S_2 .

(4) Observation that when amorphous nickel monosulfide (NiS) and nickel subsulfide (Ni_3S_2) were administered by *im* injection to randomly selected Fischer rats in equivalent amounts under identical conditions, NiS did not induce any tumors whereas Ni_3S_2 induced sarcomas in almost all of the rats.

(5) Provision of the first evidence that partially converted nickel-

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iron sulfide matte, an intermediate in nickel refining, is carcinogenic in rats.

(6) Provision of the first reported measurements of nickel in human parotid saliva, leading to the possibility that nickel analyses of parotid saliva may serve as a convenient index of human exposures to nickel.

(7) Demonstration that intrarenal injection of Ni_3S_2 in rats produces inhibition of 5'-nucleotidase activity in renal microsomes.

(8) Quantitation of fetal toxicity of Ni(II) in rats, and demonstration that $^{63}\text{Ni(II)}$ can cross the rat placenta and become localized in the fetuses.

F. William Sunderman, Jr., M.D.
August 15, 1976

ERDA RESEARCH DOCUMENT NO. COO-3140-35Progress Report for Energy Research and Development Administration Contract"The Toxicology and Metabolism of Nickel Compounds"August 15, 1976

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Scientific Accomplishments During the Past Grant Year:A. Study of Chelation Therapy of Acute Ni(II)-Toxicity.

Comparisons were made of the antidotal effectiveness of chelating drugs upon acute toxicity of Ni(II) in rats. Six chelating agents (calcium disodium versenate, d-penicillamine, Na-diethyldithiocarbamate, triethylenetetramine, reduced α -lipoic acid, and diglycylhistidine-N-methylamide) were administered to rats by *im* injection in order to compare their relative efficacies in prevention of death after a single parenteral injection of NiCl₂. Triethylenetetramine and d-penicillamine were the most effective antidotes for acute Ni(II)-toxicity. In order of decreasing antidotal effectiveness, diglycylhistidine-N-methylamide, sodium diethyldithiocarbamate and calcium disodium versenate significantly reduced the acute mortality of rats following *ip* injection of Ni(II). α -Lipoic acid was not effective as an antidote for acute Ni(II)-toxicity. In order to perform these studies, it was necessary to determine the acute lethal dosages of NiCl₂ and triethylenetetramine in rats following administration by parenteral routes. The following acute mortality indices were obtained for NiCl₂ administered to rats by *ip* injection: LD₅ = 0.046 mmole/kg; LD₅₀ = 0.110 mmole/kg (S.E. $\pm$ 0.006);

$LD_{95} = 0.29$ mmole/kg; and slope-function = 1.70. The following acute mortality indices were obtained for $NiCl_2$ administered to rats by *im* injection: $LD_5 = 0.29$ mmole/kg; $LD_{50} = 0.39$ mmole/kg (S.E. ± 0.02); $LD_{95} = 0.53$ mmole/kg; and slope-function = 1.20. The following acute mortality indices were obtained for triethylenetetramine administered to rats by *ip* injection: $LD_5 = 1.60$ mmole/kg; $LD_{50} = 2.25$ mmole/kg (S.E. ± 0.07); $LD_{95} = 3.22$ mmole/kg; and slope-function = 1.22. Triethylenetetramine and d-penicillamine had not previously been tested for antidotal efficacy against acute Ni(II)-toxicity. Our finding that both of these drugs are superior to sodium diethyldithiocarbamate in antidotal effectiveness may open new avenues for the clinical therapy of acute Ni(II) poisoning. Pilot studies in our laboratory have demonstrated that triethylenetetramine is a potent antidote to acute inhalation exposure of rats to nickel carbonyl, $(Ni(CO)_4)$. Comparisons of the relative antidotal effectiveness of triethylenetetramine, d-penicillamine and sodium diethyldithiocarbamate in rats that have been exposed to inhalation of $Ni(CO)_4$ are currently in progress. A manuscript (submitted herewith) that describes our comparisons of antidotal efficacy of chelating drugs upon acute Ni(II)-toxicity in rats has just been published in *Research Communications in Chemical Pathology and Pharmacology* (1).

B. Effects of Triethylenetetramine upon Metabolism of $^{63}Ni(II)$.

As a continuation of our study of chelation therapy of acute Ni(II)-toxicity, we investigated the effects of triethylenetetramine (TETA) upon the metabolism and excretion of $^{63}Ni(II)$ in rats, and we evaluated the antidotal effectiveness of TETA upon the proteinuria, aminoaciduria and hyperglycemia that develop in rats after an injection of Ni(II). TETA

was administered *im* to rats (0.75 mmole/kg) immediately prior to $^{63}\text{NiCl}_2$ (0.068 or 0.10 mmole/kg, *ip* or *im*). TETA greatly increased the urinary excretion of ^{63}Ni and diminished the concentrations of ^{63}Ni in plasma, heart, lung and spleen of rats. The renal clearance of the ^{63}Ni -TETA complex was more than 20 times the renal clearance of non-chelated ^{63}Ni . A fraction of the circulating ^{63}Ni -TETA complex was taken up by hepatocytes and rapidly excreted in the bile. Administration of TETA markedly decreased the severity and duration of proteinuria and aminoaciduria that developed in rats after *ip* injection of NiCl_2 , but TETA did not prevent Ni(II) -induced hyperglycemia or hyperglucagonemia. A manuscript (submitted herewith) that describes our studies of TETA effects on metabolism of $^{63}\text{Ni(II)}$ has been accepted for publication in *Toxicology and Applied Pharmacology* (2).

C. Studies of Ni_3S_2 -Induced Erythrocytosis.

As described in last year's progress report, a single intrarenal injection of Ni_3S_2 in rats produces massive erythrocytosis and marked increase in total erythrocyte mass. The goals of our initial study (which was completed during the current grant year) were: (a) to establish the dose-response and time-response relationships for Ni_3S_2 -induced erythrocytosis in rats, and (b) to elucidate the pathological reactions that occur in Ni_3S_2 -treated rats at the time of maximal erythrocytosis. In the initial study, Ni_3S_2 was administered to Fischer rats by intrarenal (*ir*) injection in dosages ranging from 0.6 to 10 mg/rat. The hematocrit, erythrocyte counts and hemoglobin concentrations became significantly increased at 1 month after the injection and reached maximum values at approximately 2 months. Thereafter, the hematocrit,

erythrocyte count and hemoglobin concentrations gradually returned to control values by 7 months. The duration and magnitude of erythrocytosis were directly related to the dosage of Ni_3S_2 . To illustrate the magnitude of the observed changes, the blood hemoglobin concentration was 24.9 ± 1.2 g/dl in rats at 2 months after bilateral *ix* injection of Ni_3S_2 (10 mg/rat), versus 15.8 ± 0.5 g/dl in saline-treated controls ($P < 0.001$). Autopsy of rats killed 2 months after *ix* injection of Ni_3S_2 showed marked erythroid hyperplasia of bone marrow, and fibrotic needle tracts in renal parenchyma with localized deposits of particles of Ni_3S_2 . A manuscript (submitted herewith) that describes this initial study has been accepted for publication in *Annals of Clinical and Laboratory Science* (3).

As a continuation of these studies, we have investigated strain differences in susceptibility of rats to induction of erythrocytosis after *ix* injection of Ni_3S_2 . This study was prompted by earlier reports that there are marked differences in susceptibility of rat strains to development of rhabdomyosarcomas after an *im* injection of Ni_3S_2 . Ni_3S_2 was administered by *ix* injection (5 mg/rat) to 12 rats of each of four strains (Fischer, Long-Evans, Wistar-Lewis and NIH-Black). Blood was collected biweekly for 6 months for measurements of hematocrit and reticulocyte count. Pronounced erythrocytosis and reticulocytosis occurred in all of the rat strains, but the response in Fischer rats was significantly greater than in the other strains. To study the sequential pathological changes, 21 Fischer rats were given *ix* injections of Ni_3S_2 (10 mg/rat) and 3 rats were killed at the following intervals after injection: 1, 2, 3, 4, 5, 8 and 10 weeks, together with control

rats that had received intrarenal NaCl solution. Extramedullary hemato-
poiesis in the spleen was marked beginning at 1 week after the injection
and erythroid hyperplasia of the bone marrow was present from 2 to 10
weeks. The kidneys showed fibrous connective tissue proliferation around
the injection sites without extensive inflammatory reaction. This study
has established the optimal strain of rats and the most favorable experi-
mental conditions for study of Ni_3S_2 -induced erythrocytosis, and it has
formed the basis for the investigations of Ni_3S_2 effects upon erythro-
poietin production that are currently in progress. An abstract (submit-
ted herewith) that describes our study of strain differences in suscep-
tibility of rats to Ni_3S_2 -induced erythrocytosis has been published in
Annals of Clinical and Laboratory Science (4), and a manuscript describ-
ing this research will be completed within six months.

D. Nickel Carcinogenesis.

Our study of the inhibitory effect of manganese on carcinogenicity
and metabolism of nickel subsulfide was completed early in this grant
year. Most of the salient conclusions of this study were included in
last year's progress report, and hence will not be duplicated in this
document. A manuscript (submitted herewith) that describes the marked
inhibitory effect of manganese on Ni_3S_2 -induction of rhabdomyosarcomas
in Fischer rats has been published in *Cancer Research* (5).

Comparisons have been completed of the carcinogenicities of metal-
lic Ni powder (Ni), nickel subsulfide (Ni_3S_2), partially converted
nickel-iron sulfide matte (Ni-FeS) and amorphous nickel monosulfide (NiS)
following intramuscular injection in Fischer rats. This study demon-
strated marked differences in the incidences of sarcomas in Fischer rats

within 2 years after a single *im* injection of these four insoluble nickel-containing compounds. Whereas Ni_3S_2 was highly carcinogenic, amorphous NiS did not induce any tumors. The carcinogenic potency of partially converted nickel-iron sulfide matte was less than Ni_3S_2 but greater than Ni powder. No sarcomas occurred at the injection site in two groups of control rats that received *im* injections of penicillin or Fe powder. The observed differences in carcinogenic potencies of Ni_3S_2 and amorphous NiS may provide an experimental approach to elucidate the molecular mechanisms of nickel carcinogenesis. A paper describing these findings was presented at the 1976 Annual Meeting of the American Association for Cancer Research (6) (abstract submitted herewith), and our manuscript (submitted herewith) on this work has been published in *Research Communications in Chemical Pathology and Pharmacology* (7).

As noted in last year's renewal proposal, carcinogenesis studies are in progress in Syrian hamsters to determine whether or not epidermoid cancers can be induced in the mucosa of the oropharynx by topical administration of Ni_3S_2 . The Ni_3S_2 was suspended in glycerol in concentrations of 0.5, 1.0, 2.5 and 5% and was applied to the mucosa of the cheek pouches of the hamsters three times weekly for 36 weeks. This two-year carcinogenesis study will not be terminated until 3/24/77. To date, no tumors of the oropharynx have developed in the four groups of 15 hamsters that received the buccal applications of Ni_3S_2 . No tumors have developed in the oropharynx of vehicle-treated control hamsters. Carcinomas of the oropharynx have developed in all of the hamsters in a second control group that received topical applications of dimethylbenzanthracene (0.5% in glycerol). In another two-year carcinogenesis

study that will not be completed until 3/24/77, four groups of 12 to 15 Syrian hamsters were given weekly intratracheal insufflations of 2 to 10 mg of Ni_3S_2 for 18 weeks. Control hamsters received insufflations of the saline vehicle. To date, none of these Ni_3S_2 -treated hamsters has developed carcinoma of the lung.

E. Measurements of Nickel in Parotid Saliva by Atomic Absorption.

Specimens of parotid saliva were obtained from 38 healthy adults with precautions to minimize nickel contamination. Analyses of nickel by electrothermal atomic absorption spectrometry showed that the mean concentration of nickel in parotid saliva was $1.9 \pm 1.0 \mu\text{g Ni/liter}$ (range = 0.8 to 4.5). There was no significant correlation between the concentrations of salivary nickel and protein. No significant differences were observed between the mean concentrations of nickel in saliva samples from (a) men vs women; (b) health center employees vs naval recruits; (c) caries-free subjects vs caries-prone subjects; or (d) non-smokers vs cigarette smokers. The Lashley technique for collection of parotid saliva was found to be relatively rapid, inoffensive, non-invasive and practical to perform with precautions against nickel contamination. For these reasons, measurements of nickel in parotid saliva may be valuable for monitoring occupational exposures to nickel. A manuscript (submitted herewith) that describes this study is in press in *Annals of Clinical and Laboratory Science* (8).

F. Effects of Nickel upon 5'-Nucleotidase in Serum and Tissues.

Measurements of 5'-nucleotidase activity in human and rat serums were performed by the method of Persijn and van der Slik (9) using

reagents that had been purified and glassware that had been scrupulously cleaned in order to minimize nickel contamination. Under these conditions, the lowest concentration of Ni(II) that consistently produced inhibition (> 10%) of 5'-nucleotidase activity in rat and human serums was 1×10^{-4} mole/liter, equivalent to 6 mg Ni/liter. From this study, it was apparent that measurements of serum 5'-nucleotidase activity are not sufficiently sensitive to nickel inhibition in order to serve as a biochemical index of occupational or environmental exposures to nickel. On the other hand, it appeared likely that the amounts of Ni_3S_2 that have been injected parenterally in experimental animals in our carcinogenesis and erythrocytosis experiments would be sufficient to cause inhibition of 5'-nucleotidase activity. To test this hypothesis, measurements of 5'-nucleotidase activity were performed by the method of Segal and Brenner (10) upon microsomes from kidneys of rats that were killed at intervals of 1, 2 or 7 days after intrarenal injection of Ni_3S_2 (5, 10 or 20 mg/kidney). The technique for intrarenal injection of Ni_3S_2 was identical to that employed in our studies of Ni_3S_2 -induction of erythrocytosis (3). The results of this study are summarized in Table 1. *In vitro* addition of Ni_3S_2 (20 mg) to renal homogenates did not inhibit microsomal 5'-nucleotidase activity, owing to the very low solubility of Ni_3S_2 . In comparison to the control values obtained in renal microsomes from rats in control groups, rats that received Ni_3S_2 by *ip* injection in dosages of 4, 5, 10 or 20 mg/rat had significant inhibition of 5'-nucleotidase activities at 1 and 2 days after the injection. In rats that received 20 mg of Ni_3S_2 , inhibition of 5'-nucleotidase activity in renal microsomes was also found at 7 days after the injection. The Ni_3S_2 inhibition of 5'-nucleotidase activity was not prevented by simultaneous intrarenal

Table 1

Measurements of 5'-Nucleotidase Activity in Microsomes from Rat Kidney

Group	Number of Rats	Treatments and Dosages	Days Between Treatment and Sacrifice	Microsomal Protein Conc. (mg prot./ g kidney) ^a	5'-NT Activity (units/mg of microsomal protein) ^a
A	19	Controls (no treatment)	-	10.4 ± 1.0	0.80 ± 0.16
B	11	Controls (0.4 ml NaCl Sol'n, <i>ir</i>) ^b	1	9.8 ± 1.0	0.74 ± 0.17
C	15	Controls (0.4 ml NaCl Sol'n, <i>ir</i>)	2	10.3 ± 0.7	0.83 ± 0.17
A-C	45	All Controls	-	10.2 ± 1.0	0.80 ± 0.17
D	10	Ni ₃ S ₂ , 20 mg, <i>in vitro</i> ^c	-	11.0 ± 0.9	0.83 ± 0.21
E	11	Ni ₃ S ₂ , 5 mg, <i>ir</i>	1	9.5 ± 0.9	0.60 ± 0.11 ^e
F	10	Ni ₃ S ₂ , 10 mg, <i>ir</i>	1	10.7 ± 0.7	0.66 ± 0.17 ^d
G	10	Ni ₃ S ₂ , 20 mg, <i>ir</i>	1	9.1 ± 0.8 ^d	0.38 ± 0.07 ^e
H	15	Ni ₃ S ₂ , 5 mg, <i>ir</i>	2	9.9 ± 0.8	0.62 ± 0.15 ^e
I	10	Ni ₃ S ₂ , 10 mg, <i>ir</i>	2	10.9 ± 0.7	0.46 ± 0.20 ^e
J	15	Ni ₃ S ₂ , 20 mg, <i>ir</i>	2	10.4 ± 0.6	0.34 ± 0.09 ^e
K	9	Ni ₃ S ₂ , 20 mg, <i>ir</i>	7	9.8 ± 1.2	0.52 ± 0.12 ^e
L	10	Mn, 8.8 mg, <i>ir</i>	2	11.3 ± 1.0 ^d	0.63 ± 0.10 ^e
M	10	Ni ₃ S ₂ , 10 mg & Mn, 8.8 mg, <i>ir</i>	2	10.7 ± 0.6 ^d	0.30 ± 0.09 ^e

^a mean ± standard deviation.

^b intrarenal injection (*ir*) of 0.2 ml of NaCl solution, (145 mmole/liter) into both poles of right kidney. This NaCl solution was the vehicle for all *ir* injections.

^c Ni₃S₂, 20 mg. added *in vitro* to the renal homogenate before sedimentation of nuclei and mitochondria.

^d P < 0.05 vs all controls (group A to C).

^e P < 0.001 vs all controls (group A to C).

injection of manganese powder (8.8 mg). A manuscript describing our investigations of nickel inhibition of 5'-nucleotidase activities in serum and in renal microsomes is now being drafted and will soon be submitted for publication in *Annals of Clinical and Laboratory Science*.

G. Fetal Toxicity and Teratogenesis of Nickel Compounds.

NiCl_2 was administered *im* to pregnant Fischer rats on the eighth day of gestation in dosages ranging from 8 to 16 mg Ni/kg body weight. The dams were killed on the twentieth day of gestation, and the number of corpora lutea, conceptuses and fetuses was determined, and the products of conception were examined. One half of the fetuses were fixed in formalin and dissected under the dissecting microscope to identify visceral anomalies. The remaining fetuses were clarified with glycerol and stained with alizarin red to reveal abnormalities of the skeleton. The results of this study are summarized on Table 2. NiCl_2 administration produced a dose-related reduction in the average number of live fetuses/dam, and a dose-related increase in the ratio of resorbed or dead fetuses to the total number of conceptuses. No fetal malformations were found in the saline treated controls or in any of the NiCl_2 -treated rats. Also shown in Table 2 are the results that were obtained when NiCl_2 was administered to pregnant dams in a total dosage of 15 mg/kg body weight as 10 divided doses of 1.5 mg/kg *bid*, on the 6th to 10th day of gestation. Administration of Ni_3S_2 (8 mg Ni/kg body weight, suspended in 0.2 ml of penicillin) *im* on the 6th day of gestation caused a significant reduction in the average number of live fetuses/dam, and a significant increase in the ratio of dead or resorbed fetuses to the total number of conceptuses. No fetal malformation were found in the penicillin-treated controls or in the Ni_3S_2 -treated rats. These studies

Table 2

Studies of Fetal Toxicity of Nickel Compounds in Rats^a

Group	No. of Pregnant Dams	Treatments and Dosages	Gestational Days of Treatment	No. of Corpora Lutea ($\bar{x} \pm S.D.$)	No. of Conceptuses ($\bar{x} \pm S.D.$)	No. of Live Fetuses ($\bar{x} \pm S.D.$)	Wt. of Live Fetuses ($\bar{g} \pm S.D.$)	Ratio of Conceptuses to Corpora Lutea	Ratio of Dead Fetuses to Conceptuses
A	13	Controls (0.4 ml NaCl sol'n, im) ^b	8	9.92 $\pm$ 1.14	9.85 $\pm$ 1.17	9.69 $\pm$ 1.14	3.45 $\pm$ 0.26	128/129 (99.2%)	2/128 (1.6%)
B	14	Controls (0.2 ml penicillin, im) ^c	6	11.00 $\pm$ 1.13	10.57 $\pm$ 1.24	10.50 $\pm$ 1.24	3.17 $\pm$ 0.26	145/154 (96.1%)	1/148 (0.7%)
A & B	27	All Controls	6 or 8	10.48 $\pm$ 1.26	10.22 $\pm$ 1.26	10.11 $\pm$ 1.26	3.30 $\pm$ 0.29	276/283 (97.5%)	3/276 (1.1%)
C	12	NiCl ₂ (8 mg Ni/kg, im)	8	10.08 $\pm$ 0.95	9.42 $\pm$ 1.71	8.92 $\pm$ 2.02	3.43 $\pm$ 0.36	113/121 (93.4%)	6/113 (5.3%) ^g
D	11	NiCl ₂ (12 mg Ni/kg, im)	8	9.73 $\pm$ 0.96	8.45 $\pm$ 1.56 ^g	7.73 $\pm$ 1.91 ^f	3.19 $\pm$ 0.43	93/107 (86.9%) ⁱ	8/93 (8.3%) ⁱ
E	12	NiCl ₂ (16 mg Ni/kg, im)	8	10.67 $\pm$ 1.18	8.58 $\pm$ 2.69	7.00 $\pm$ 3.87 ^c	3.03 $\pm$ 0.32 ^d	103/128 (80.5%) ⁱ	19/103 (18.5%) ⁱ
F	12	NiCl ₂ (1.5 mg Ni/kg, im, bid) ^d	6 to 10	9.75 $\pm$ 1.48	9.58 $\pm$ 1.80	9.42 $\pm$ 1.80	3.33 $\pm$ 0.30	115/119 (96.6%)	2/115 (1.7%)
G	12	Ni ₃ S ₂ (8 mg Ni/kg, im)	6	9.67 $\pm$ 1.65	8.83 $\pm$ 2.37	7.75 $\pm$ 2.89 ^d	3.16 $\pm$ 0.38	106/116 (91.4%) ^h	13/106 (12.3%) ⁱ

^a Pregnant dams were killed on the 20th day of gestation.

^b Intramuscular injection of 0.4 ml of NaCl solution (145 mmole/liter). This NaCl solution was also the vehicle for injections of NiCl₂ in Groups C-F.

^c Intramuscular injection of 0.2 ml of penicillin G procaine suspension (100,000 units/ml). This penicillin solution was also the vehicle for injections of Ni₃S₂ in Group G.

^d P < 0.02 vs all controls (Group A & B) by "t" test.

^e P < 0.01 vs all controls (Groups A & B) by "t" test.

^f P < 0.001 vs all controls (Groups A & B) by "t" test.

^g P < 0.05 vs all controls (Groups A & B) by " χ^2 " test.

^h P < 0.02 vs all controls (Groups A & B) by " χ^2 " test.

ⁱ P < 0.001 vs all controls (Groups A & B) by " χ^2 " test.

demonstrate that administration of NiCl_2 or Ni_3S_2 to pregnant rats in dosages that did not produce maternal mortality were sufficient to produce fetal mortality. There are obvious practical implications of these findings in respect to potential fetal hazards from nickel exposures of pregnant women who are employed in nickel industries.

Radioactive $^{63}\text{NiCl}_2$ was administered *im* in dosage of 12 mg Ni/kg body weight to (a) non-pregnant female rats, (b) pregnant rats on the 8th day of gestation, and (c) pregnant rats on the 18th day of gestation. This dosage was selected on the basis of a pilot study (Table 3) which showed that administration of NiCl_2 in dosage of 12 mg Ni/kg body weight *im* on the 18th day of gestation did not produce any mortality of fetal rats within 24 hr after injection. At 24 hours after the injection of $^{63}\text{NiCl}_2$, the rats were killed and several organs and tissues were obtained for measurements of ^{63}Ni by liquid scintillation spectrometry. The results are summarized in Table 4. In order of decreasing concentrations of ^{63}Ni per g of tissue (wet wt), the relative localization of ^{63}Ni in organs and tissues of non-pregnant and pregnant rats was: kidneys > serum > adrenal = lungs = ovary > spleen = heart = liver > skeletal muscle. A notable finding was that the mean concentrations of ^{63}Ni in pituitaries from pregnant rats on the 9th and 19th days of gestation were 7 to 8 times greater than in the pituitaries from non-pregnant rats. The concentrations of ^{63}Ni in the products of conception (placentas and fetuses) on the 9th day of gestation were approximately the same as in the adrenals, lungs and ovaries from these rats, and were much higher than in spleen, liver, heart or skeletal muscle. The mean concentration of ^{63}Ni in placentas from rats on the 19th day of gestation was second only to the

Table 3

Studies of Fetal Toxicity of NiCl_2 in Rats on the 19th Day of Gestation^{a,b}

<u>Group</u>	<u>No. of Pregnant Dams</u>	<u>Treatments and Dosages</u>	<u>No. of Conceptuses ($\bar{x} \pm S.D.$)</u>	<u>No. of Live Fetuses ($\bar{x} \pm S.D.$)</u>	<u>Ratio of Dead Fetuses to Conceptuses</u>
H	12	Controls (0.4 ml NaCl sol'n, <i>im</i>) ^c	9.92 ± 0.85	9.83 ± 0.77	1/119 (0.8%)
I	10	NiCl_2 (6 mg Ni/kg, <i>im</i>)	9.60 ± 1.42	9.60 ± 1.42	0/96 (0.0%)
J	12	NiCl_2 (8 mg Ni/kg, <i>im</i>)	8.55 ± 2.15	8.55 ± 2.15	0/104 (0.0%)
K ^d	15	NiCl_2 (16 mg Ni/kg, <i>im</i>)	9.67 ± 1.54	7.92 ± 4.50	29/145 (20.0%) ^e

^a Pregnant dams were killed on the 19th day of gestation, at 24 hr after *im* injection of NaCl or NiCl_2 .

^b The acute (2 week) toxicity indices of NiCl_2 administered to pregnant Fischer rats on the 18th day of gestation were: $\text{LD}_5 = 13$ mg Ni/kg; $\text{LD}_{50} = 16$ (S.E. ± 0.03) mg Ni/kg; $\text{LD}_{95} = 19$ mg Ni/kg; slope function = 1.12. For comparison the acute (2 week) toxicity indices of NiCl_2 administered to non-pregnant, female Fischer rats were: $\text{LD}_5 = 17$ mg Ni/kg; $\text{LD}_{50} = 22$ (S.E. ± 0.02) mg Ni/kg; $\text{LD}_{95} = 27$ mg Ni/kg; slope function = 1.16.

^c Intramuscular injection of 0.4 ml of NaCl solution (145 mmole/liter). This NaCl solution was also the vehicle for injections of NiCl_2 in Groups I to K.

^d Dams that died within 24 hr after *im* injection of NiCl_2 (16 mg Ni/kg), were excluded from Group K.

^e $P < 0.001$ vs controls (Group H) by " χ^2 " test.

Table 4

Concentrations of ^{63}Ni in Organs and Tissues of Pregnant and Non-Pregnant Rats^a after Intramuscular Injection of $^{63}\text{NiCl}_2$ ^b

Organ or Tissue	^{63}Ni Concentration ($\mu\text{g/g}$ wet wt.) ^d		
	Non-Pregnant	Pregnant Rats	
	♀ Rats	9th Day ^d	19th Day ^d
Kidney	16.35 $\pm$ 3.13	12.22 $\pm$ 2.65	9.64 $\pm$ 1.71 ^g
Serum	3.67 $\pm$ 0.48	3.17 $\pm$ 0.61	1.79 $\pm$ 0.10 ^h
Adrenal	2.92 $\pm$ 0.47	1.89 $\pm$ 0.39	1.49 $\pm$ 0.27 ^h
Lung	2.32 $\pm$ 0.70	1.95 $\pm$ 0.30	2.04 $\pm$ 0.27
Ovary	2.24 $\pm$ 1.07	1.97 $\pm$ 0.59	0.91 $\pm$ 0.25 ^f
Uterus	2.07 $\pm$ 0.26	-	-
Spleen	0.78 $\pm$ 0.20	0.62 $\pm$ 0.26	0.70 $\pm$ 0.19
Heart	0.71 $\pm$ 0.12	0.46 $\pm$ 0.10	0.61 $\pm$ 0.19
Liver	0.57 $\pm$ 0.30	0.62 $\pm$ 0.19	0.44 $\pm$ 0.06
Skeletal Muscle	0.19 $\pm$ 0.03	0.16 $\pm$ 0.03	0.13 $\pm$ 0.02
Pituitary	0.13 $\pm$ 0.05	1.09 $\pm$ 0.25 ^h	0.91 $\pm$ 0.24 ^h
Placenta & Fetuses ^e	-	2.00 $\pm$ 0.38	-
Placenta	-	-	2.61 $\pm$ 0.65
Fetuses	-	-	1.18 $\pm$ 0.43
Amniotic Fluid	-	-	0.62 $\pm$ 0.21

^a N = 12: 4 rats in each group

^b 12 mg Ni/kg body wt., administered *im* as $^{63}\text{NiCl}_2$ at 24 hr before death.

^c Mean $\pm$ standard deviation.

^d Day after observation of sperm in vagina.

^e Uterine contents (*i.e.* products of conception).

^f P < 0.05 *vs* values in non-pregnant rats, computed by "t" test.

^g P < 0.01 *vs* values in non-pregnant rats, computed by "t" test.

^h P < 0.001 *vs* values in non-pregnant rats, computed by "t" test.

mean concentration of ^{63}Ni in the kidneys, and was higher than the mean concentrations of ^{63}Ni in all of the other organs and tissues that were studied. Appreciable concentrations of ^{63}Ni were also found in the fetuses and amniotic fluid from rats on the 19th day of gestation. These findings demonstrate that $^{63}\text{Ni}(\text{II})$ can cross the rat placenta and become localized in the fetuses. A manuscript describing these studies of fetal toxicity and teratogenicity of nickel compounds should be drafted within 6 months. for publication in *Toxicology and Applied Pharmacology*.

H. Miscellaneous Accomplishments.

A review article (submitted herewith) on the carcinogenicities of nickel, chromium and arsenic compounds in man and animals was written upon the invitation of Dr. Phillipe Shubik, and was included in a symposium on occupational carcinogenesis that was published in *Preventive Medicine* (11).

The principal investigator was invited to participate in a Working Party on Nickel Carcinogenesis under the auspices of the International Agency for Cancer Research (Lyon, France). The principal investigator drafted a manuscript (submitted herewith) on Evaluation of Carcinogenic Risk to Man from Exposure to Nickel Compounds which was thoroughly discussed and reviewed by the Working Party. The manuscript has been published in an IARC Monograph (12).

The principal investigator has been selected by the Commission on Toxicology of the International Union of Pure and Applied Chemistry to be Chairman of the Subcommittee on Environmental and Occupational Toxicology of Nickel. This Subcommittee includes toxicologists from the

United States, Canada, England, Norway, France, Germany, Japan, and Poland, and it has already sponsored three interlaboratory comparisons of nickel analyses in body fluids. Within the next year, the Nickel Subcommittee plans to draft an "International Reference Method for Analysis of Nickel in Biological Materials by Atomic Absorption Spectrometry." The efforts of this panel should promote international standardization and cooperation in the field of nickel toxicology.

The principal investigator was invited to present a review paper on normal values at the Second Aspen Conference on Clinical Pathology. The manuscript (submitted herewith) has been published in *Clinical Chemistry* (13).

I. Compliance with Contract Requirements.

The specific objectives of this year's research, as specified in last year's renewal proposal were:

(1) Study of Chelation Therapy of Acute Nickel Poisoning. Comparisons of the antidotal effectiveness of several chelating drugs in acute Ni(II) toxicity have been completed, as specified in Section A of this Progress Report. Studies of the effect of triethylenetetramine (TETA) on the metabolism and excretion of $^{63}\text{NiCl}_2$ have been completed, as specified in Section B of this Progress Report. Evaluations of the antidotal effectiveness of TETA, d-penicillamine and Na-diethyldithiocarbamate in acute $\text{Ni}(\text{CO})_4$ poisoning by inhalation exposure are currently underway.

(2) Study of Ni_3S_2 -Induced Erythrocytosis. Studies of the dose-response and time-response relationships for induction of erythrocytosis in rats after intrarenal injection of Ni_3S_2 have been completed, as specified in Section C of this Progress Report. Studies of (a) differences in

susceptibilities of rat strains to Ni_3S_2 -induced erythrocytosis, (b) rates of excretion of ^{63}Ni in urine after intrarenal injection of Ni_3S_2 , and (c) serial sacrifice study of pathological reactions to intrarenal Ni_3S_2 are nearing completion. Investigations of the role of erythropoietin in Ni_3S_2 -induced erythrocytosis have been begun in collaboration with Dr. Albert Gordon of New York University.

(3) Carcinogenesis studies. The phases of this project that were scheduled to be undertaken during the past year have been completed, as specified in Section D of this Progress Report.

(4) Development of Biochemical Indices of Nickel Exposure. Studies of effects of intrarenal injection of Ni_3S_2 on renal activities of 5'-nucleotidase, and studies of the effect of Ni(II) upon serum activity of 5'-nucleotidase are nearing completion, as specified in Section F of this Progress Report. In addition, measurements have been made of nickel concentrations in parotid saliva, as specified in Section E of this Progress Report.

(5) Gas Chromatographic Detection of Volatile Nickel Compounds in Blood and Breath. Work on this project is currently in progress. To date, no major improvements have been accomplished in the gas chromatographic method for measurement of Ni(CO)_4 in blood and breath. The method is being used for study of the effects of triethylenetetramine (TETA) upon the inhalation toxicity of Ni(CO)_4 in rats.

(6) Studies of Fetal Toxicity, Teratogenicity and Mutagenicity of Nickel Compounds in Rats. As described in Section G of this Progress Report, significant progress has been made in (a) evaluating the fetal toxicity and teratogenicity of NiCl_2 in rats, and (b) studying the ability of $^{63}\text{Ni(II)}$ to cross the placenta and become localized in the fetus.

Similar studies using Ni(CO)_4 are underway. Investigations of the mutagenicity of NiCl_2 and Ni(CO)_4 by the dominant lethal mutation test will soon be initiated.

J. Effort of the Principal Investigator. The Principal Investigator devotes 25% of his time and effort to this project during the period from December 1, 1975 to June 30, 1976.

K. Significance of the Research and its Relevance to National Priorities. There is very serious national and international concern regarding environmental pollution and industrial toxicity from nickel and its compounds, as indicated (a) by the recent report on nickel by the Committee on Medical and Biological Pollutants of the National Academy of Sciences, U.S.A. (13), (14), (b) by the monograph on Carcinogenic Risk to Man from Exposure to Nickel and Inorganic Nickel Compounds which has been issued by the International Agency for Cancer Research (12) and (c) by the recent creation of the Subcommittee on Environmental and Occupational Toxicology of Nickel by the International Union of Pure and Applied Chemistry. The World Health Organization is currently drafting an Environmental Criteria Document for Nickel which will focus additional attention upon the human hazards from exposure to nickel compounds. Insofar as the Principal Investigator can ascertain, this research program at the University of Connecticut is the only research program in the United States which is specifically focused upon the toxicology and metabolism of nickel compounds. The relevance of this research to national priorities has been enhanced by the introduction of nickel catalysts in several industrial processes for gasification of coal. Formation and release of volatile nickel compounds (e.g. Ni(CO)_4) and of nickel sulfides (e.g. Ni_3S_2) may represent serious, unrecognized

hazards to industrial workers and even to the general population. Detailed discussion of the pertinence of nickel toxicology to the health related effects of coal as an energy source was included in last year's Research Proposal and hence is not repeated in this Progress Report. The data on toxicity, carcinogenicity, teratogenicity and mutagenicity of nickel compounds, and the biochemical indices and analytical methods for detection of nickel exposures that are being derived from this research program will become especially important to national priorities if nickel-catalyzed gasification technologies for coal achieve widespread adoption.

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