DEVELOPMENTAL AND REPRODUCTIVE TOXICANT IDENTIFICATION COMMITTEE (DARTIC)

October 2018 Meeting

Consideration of Nickel and Nickel Compounds for Listing Under Proposition 65 as Known to Cause Reproductive Toxicity

Reproductive Toxicology and Epidemiology Section
Office of Environmental Health Hazard Assessment

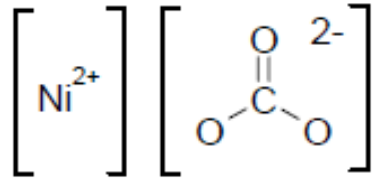
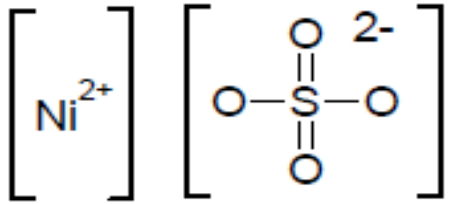
NICKEL & NICKEL COMPOUNDS

- Selected as potential candidates for consideration by application of OEHHA's "Process for Prioritizing Chemicals for Consideration under Proposition 65 by the 'State's Qualified Experts'"
- Presented to the DARTIC as potential candidates for consideration on November 9, 2015
- Recommended for consideration by the DARTIC

NICKEL & NICKEL COMPOUNDS

Metallic nickel and nickel compounds have many industrial and commercial applications, including use in stainless steel and other nickel alloys, catalysts, batteries, pigments, and ceramics

NICKEL (Ni)

$NiCO_3$	$NiCl_2$	$NiSO_4$
	Cl – Ni – Cl	
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SOLUBILITY OF NICKEL & NICKEL COMPOUNDS

Chemical	Solubility in Water
Nickel chloride ($NiCl_2$)	642,000 mg/L at 20 °C
Nickel nitrate hexahydrate ($Ni(NO_3)_2 \cdot 6H_2O$)	485,000 mg/L at 20 °C
Nickel sulfate ($NiSO_4$)	293,000 mg/L at 0 °C
Nickel acetate ($Ni(CH_3CO_2)_2$)	170,000 mg/L at 68 °C
Nickel ammonium sulfate ($Ni(NH_4)_2(SO_4)_2$	104,000 mg/L at 20 °C
Nickel subsulfide (Ni_3S_2)	517 mg/L at 37 °C
Nickel carbonate ($NiCO_3$)	93 mg/L at 25 °C
Nickel (Ni)	1.13 mg/L at 37 °C
Nickel oxide (NiO)	1.1 mg/L at 20 °C
Nickel cyanide ($Ni(CN)_2$)	Insoluble
Nickel sulfide (NiS)	Insoluble
Nickel carbonyl ($Ni(CO_4)$)	Insoluble

Note: Ni compounds in **red** have reproductive toxicity data included in the Hazard Identification Document

Modified from: Agency for Toxic Substances and Disease Registry **Toxicological Profile for Nickel** (2005)

NICKEL CARBONYL

- Nickel carbonyl was listed under Proposition 65 as known to cause reproductive toxicity (developmental endpoint) on September 1, 1996

- Listing was based on formal identification of nickel carbonyl as causing developmental toxicity by the U.S. Environmental Protection Agency, a designated Proposition 65 Authoritative Body

Human Developmental Toxicity

Epidemiologic Studies of the Developmental Toxicity of Nickel and Nickel Compounds

- Spontaneous abortion (2 studies)
- Fetal growth (10 studies)
- Congenital malformations (7 studies)
- Autism Spectrum Disorders (ASD: 7 studies)
- Transplacental carcinogenicity (3 studies)
- Other developmental effects (4 studies)

The Kola Peninsula, Russia

Spontaneous Abortion

Study	Design	Exposure Assessment	Results
Chashschin et al. 1994	Cross-sectional	Work in Ni hydrometallurgy	RR=1.8
Vaktskjold et al. 2008a	Case-control	Occupation categories within a Ni, cobalt, & copper refinery complex	ORs Questionnaire Low 1.39 (0.88, 1.19) High 1.27 (0.87, 1.86) Registry 1.10 (0.82, 1.47)

Fetal Growth Parameters

10 studies examined exposure to Ni as a risk factor for fetal growth restriction, as indicated by:

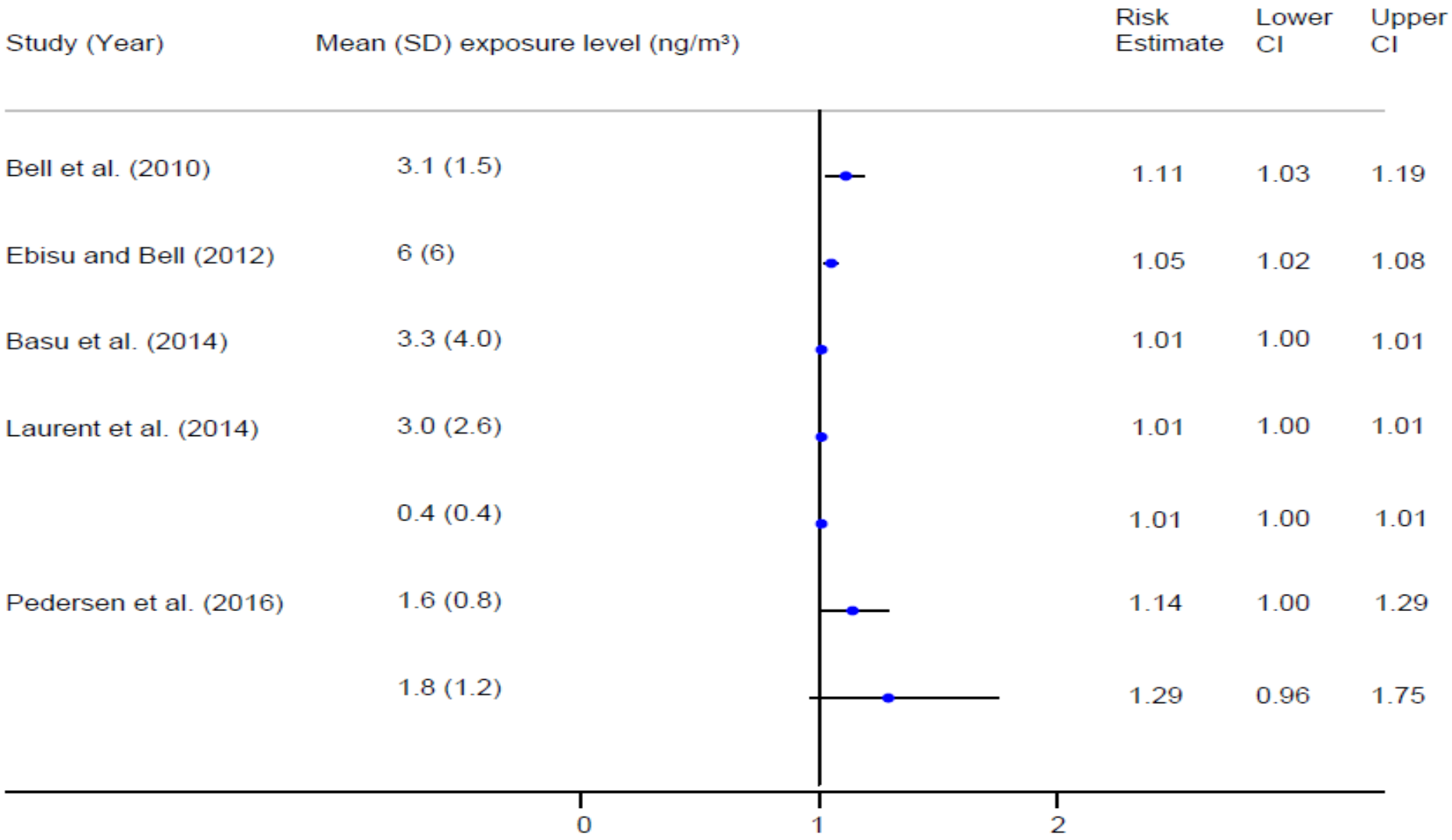
- Birth weight
- Low birth weight (LBW: birth weight < 2,500 g)
- Small for gestational age (SGA)
- Body mass index of child (BMIC)
- Head circumference

Ni exposures were measured in maternal and cord blood and urine, placenta, air pollution, soil, and by refinery occupation category

Fetal Growth Studies with Measurement of Ni in Biological Samples

Study	Design	Exposure Assessment	Results
Odland et al. 1999 **Odland et al. 2004**	Cross-sectional 3 cities in each Russia & Norway	Maternal blood and urine, infant urine, placenta (2004)	Placenta Ni and infant weight: β (CI) = -1510 (-3191, 170) g per µg/g; effect appeared smaller in multivariate analyses.
Hu et al. 2015	Not described; pilot	Maternal blood, umbilical cord blood (UCB)	Maternal blood Ni and BW β (CI) = 45.6 (-17.2, 108.4) UCB Ni and BW β (CI) = 32.2 (-19.8, 84.1)

Fetal Growth and Air Pollution



Other Fetal Growth Studies

Study	Exposure Assessment	Results
Vaktskjold et al. 2007	Occupation category	OR for SGA per unit increase in exposure category 0.84 (0.75 – 0.93).
McDermott et al. 2014	Kriged Ni conc. in soil: 4.58 mg/kg for LBW, 4.57 mg/kg for normal weight births; IQR 43.21 mg/kg	OR for LBW: 1.00 (0.98, 1.02) per IQR increase in Ni

Congenital Malformations

Outcomes

- Any birth defects
- Neural tube defects
- Genital malformations
- Musculoskeletal defects
- Cardiovascular defects

Exposure assessment

- Occupational (Ni refinery)
- Soil
- Fetal tissues
- Newborn hair samples

Congenital Malformations

Study	Exposure Assessment	Results
Chashschin et al. 1994	Work in Ni hydrometallurgy	RRs for Ni vs. construction work: All structural malformations 2.9 Cardiovascular defects 6.1 Musculoskeletal defects 1.9
Vaktskjold et al. 2006 **Vaktskjold et al. 2008b**	Occupation-based exposure categories	ORs (CI) (vs. background): Genital malformations 0.81 (0.52, 1.26) Undescended testes 0.76 (0.40, 1.47) Musculoskeletal defect 0.96 (0.76 – 1.21)

Congenital Malformations (cont'd)

Study	Exposure Assessment	Results
Huang et al. 2011	Soil samples from each village	"Layered level effects" of Ni on prevalence of NTDs Ni conc. (µg/g) — NTD prevalence <30 — high 30 – 34 — low >34 — medium
Zheng et al. 2012	Samples of soil used for food cultivation in each village	Ni conc. (µg/g) — β — RR < 37.54 — (ref) — (ref) 37.54 – 41.04 — –0.39 — 0.67 41.04 – 41.86 — –0.62 — 0.54 > 44.86 — –0.83 — 0.44

Congenital Malformations (cont'd)

Study	Exposure Assessment	Results
Friel et al. 2005	Fetal liver, kidney, sciatic nerve, pancreas, muscle Range: 1.6 (liver) – 36 (sciatic nerve) ppm	No differences between anencephalic and control fetuses
Manduca et al. 2014	Newborn hair Ni concentrations not reported	No differences between birth defects cases and normal births

Autism Spectrum Disorder (ASD)

Study	Ni Concentration (ng/m^3)	Odds Ratios
Windham et al. 2006	Mean ± SD: cases 4.3 ± 5.9; controls 3.7 ± 3.8	1.46 (1.04, 2.06)
Kalkbrenner et al. 2010	Geometric mean ± SD: North Carolina 1.1 ± 2.0; West Virginia 0.2 ± 6.3	1.1 (0.6, 1.9)
Roberts et al. 2013	1st quintile median 0.4 5th quintile median 15.9	1.65 (1.10, 2.47)
McCanlies et al. 2012	Not measured (occupational)	1.3 (0.6, 3.3); estimated from graph
von Ehrenstein et al. 2014	Mean ± SD 6.39 ± 2.25; IQR 1.82	0.97 (0.89, 1.05)
Talbott et al. 2015	IQR: cases 0.27; controls 0.20	0.76 (0.44, 1.31)
Kalkbrenner et al. 2018	Mean 1.9, IQR 1.5	1.08 (0.77, 1.51)

Transplacental Carcinogenicity

Study	Exposure Assessment	Odds Ratios
Heck et al. 2013 **Heck et al. 2015**	Air toxics data from California Air Resources Board, nearest monitor	Neuroblastoma 1.08 (0.71, 1.66) (5 km) 0.67 (0.29, 1.56) (2.5 km) Retinoblastoma 1.48 (1.08, 2.01)
Togawa et al. 2016	Parental occupation: Any Ni exposure Exposure index	Testicular germ cell tumors Paternal 1.07 (1.00, 1.16) Maternal 1.07 (0.74, 1.51) Paternal 1.00 (0.96, 1.04) Maternal 1.09 (0.91, 1.31)

Biochemical Effects

Study	Exposure Assessment	Results
Ni et al. 2014	Umbilical cord blood (UCB)	8-hydroxydeoxyguanosine (8-OHdG) as a marker of oxidative DNA damage β = 0.215 (95% CI 0.113 – 0.317)

Animal Developmental Toxicity

Animal Studies of Developmental Toxicity

- Oral Route
  - Rats: 8 studies
  - Mice: 7 studies
- Inhalation Route
  - Rats: 1 study
- Injection Routes
  - Intraperitoneal (ip)
    - Rats: 1 study
    - Mice: 3 studies
  - Intramuscular (im)
    - Rats: 1 study
  - Subcutaneous (sc)
    - Rats: 1 study
  - Intrarenal
    - Rats: 1 study
  - Intravenous (iv)
    - Hamsters: 1 study

Studies Conducted in Rats by the Oral Route

Teratology Study

- *Adjroud 2013*
  - No significant adverse fetal effects at 20 mg $NiCl_2$/L drinking water

One-generation Reproduction Studies

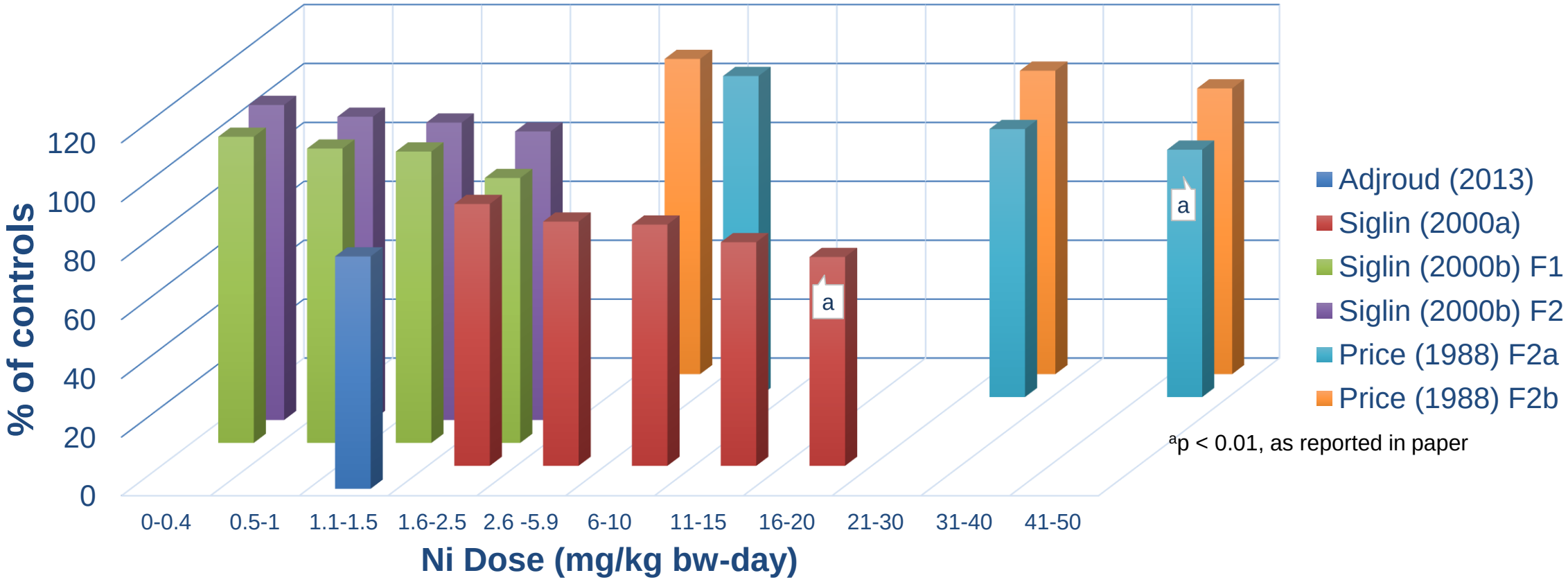
- *Siglin 2000a (Springborn, NIPERA)*
  - Fetal LOAEL 2.2 mg Ni/kg-day by gavage
- *Kakela et al. 1999*
  - No clear effect of gestational exposure from drinking water exposure
- *Smith et al. 1993*
  - Perinatal LOAEL 1.3 mg Ni/kg-bw in drinking water
- *Schroeder and Mitchener 1971*
  - Significant adverse fetal effects at 5 ppm in drinking water

Two- or Multi-generation Reproduction Studies

- *Siglin 2000b (Springborn, NIPERA)*
  - Fetal NOAEL 2.2 mg Ni/kg-day by gavage
- *Price et al. 1988 (RTI)*
  - Significant adverse fetal effects at 500 ppm in drinking water
- *RTI, 1987 as cited by US EPA, 1991a*
  - Significant adverse fetal effects at 500 ppm in drinking water
- *Ambrose et al. 1976*
  - Unclear adverse fetal effects in a feeding study

Oral Exposure in Rats: Live Litter Size as % of Controls

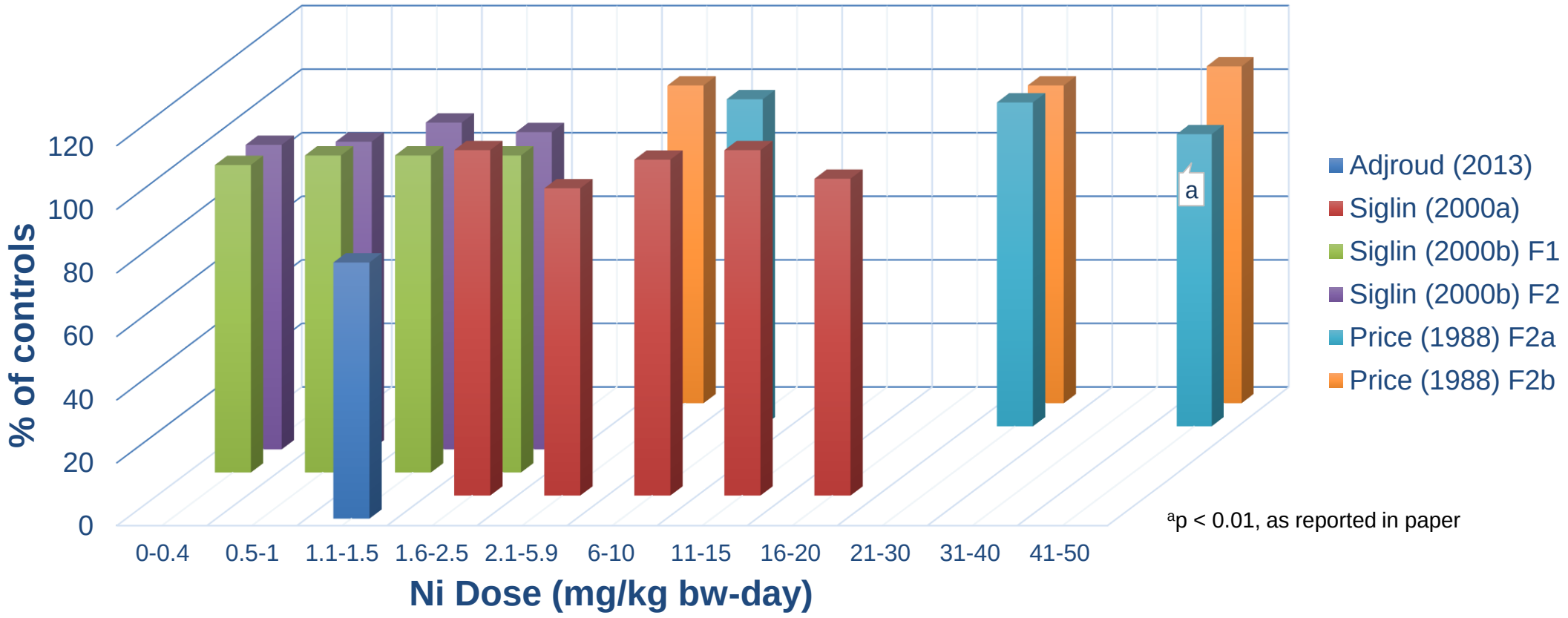
($NiSO_4$ for Siglin 2000 a & b; all others $NiCl_2$)



[a]p < 0.01, as reported in paper

Oral Exposure in Rats: Fetal/Birth Weight as % of Controls

($NiSO_4$ for Siglin 2000 a & b; all others $NiCl_2$)

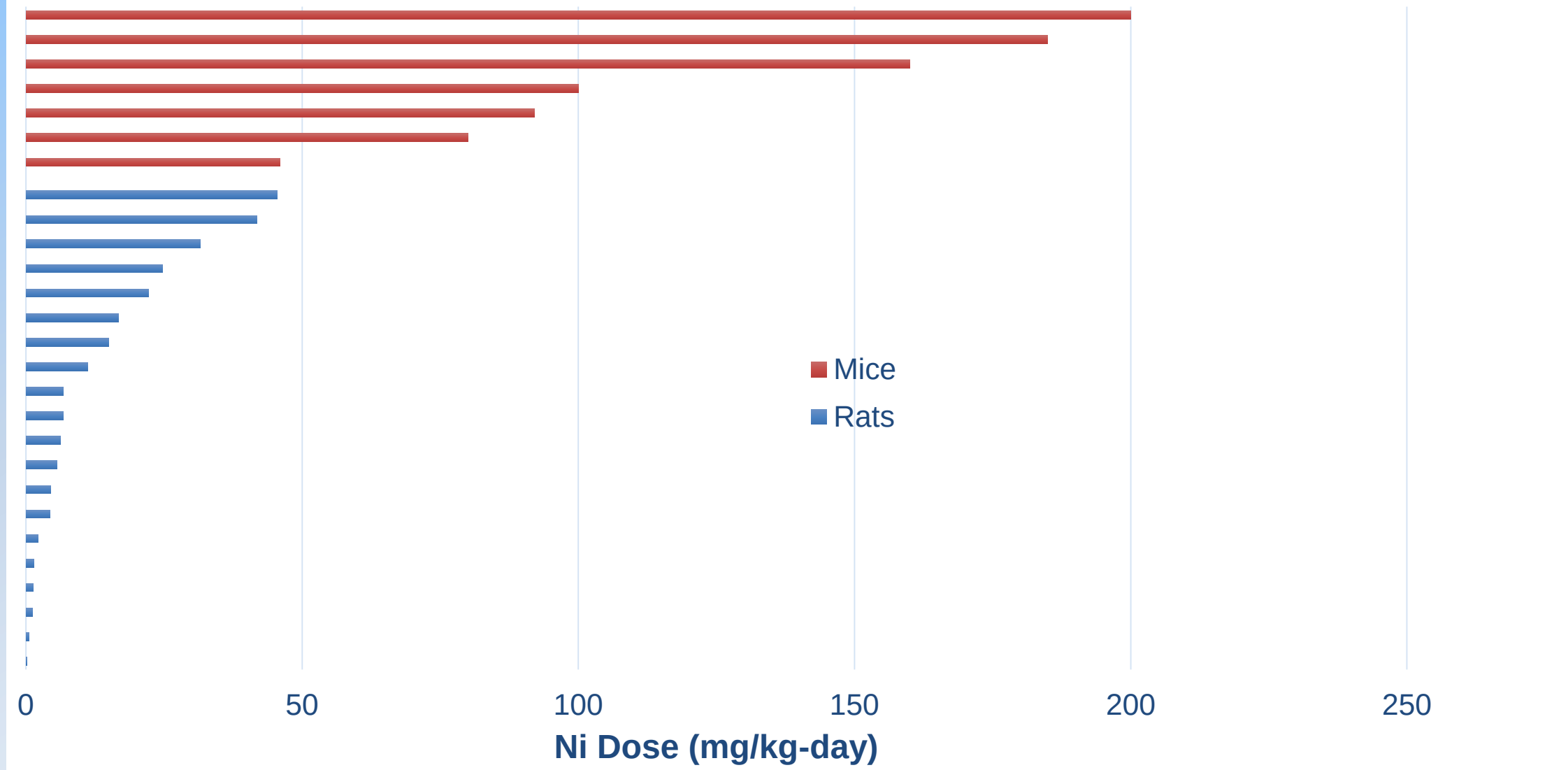


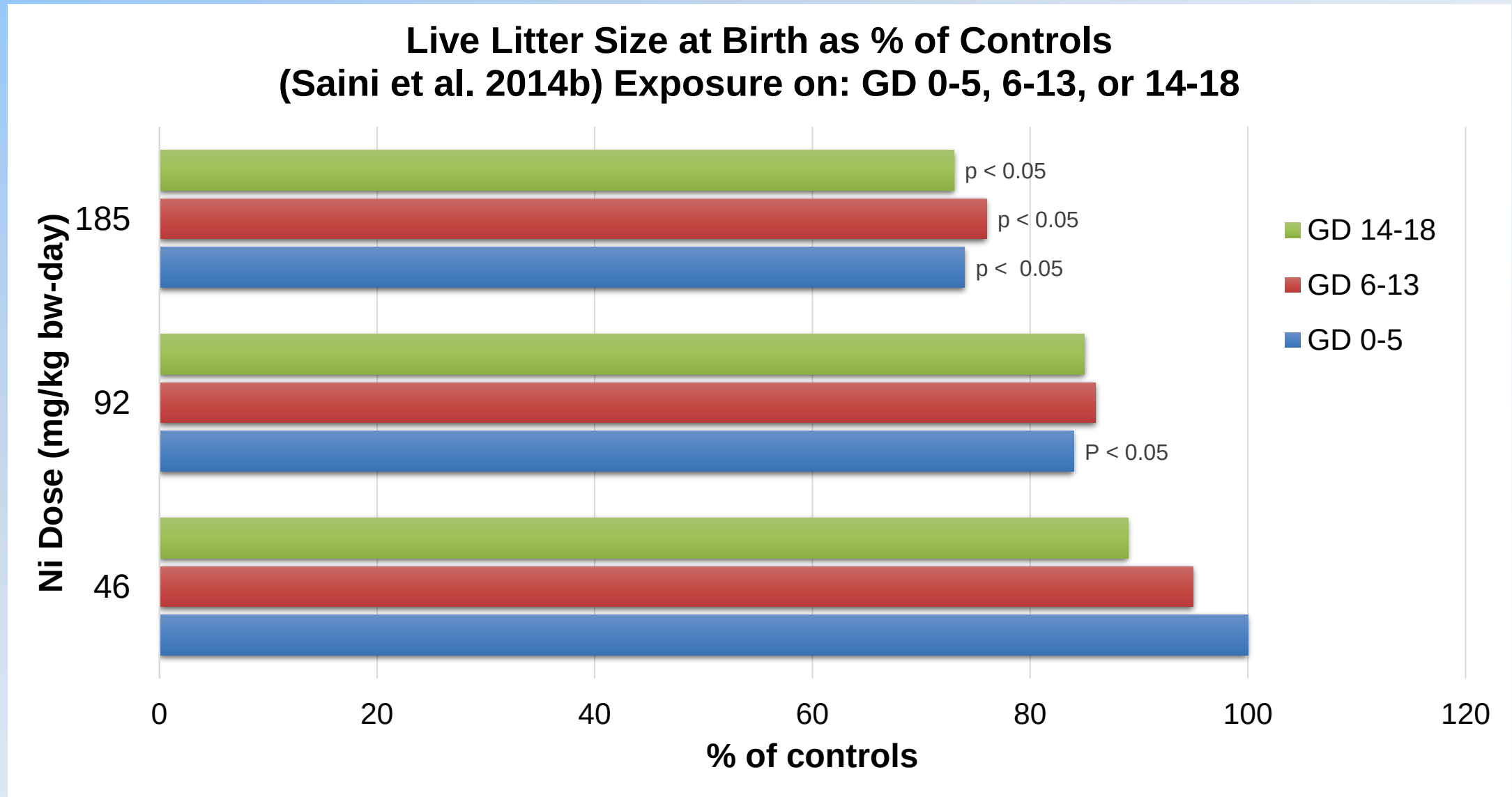
[a]p < 0.01, as reported in paper

Studies Conducted in Mice by the Oral Route

- *Saini et al. 2014a*
  - Significant adverse fetal effects at 46 mg/kg-day and above; preimplantation exposure
- *Saini et al. 2014b*
  - Adverse fetal effects depended on dose and timing of exposure
- *Saini et al. 2013*
  - Significant adverse fetal effects at 46 mg Ni/kg-day or more; organogenesis exposure
- *Berman and Renberg, 1983*
  - Unclear adverse fetal effects; gestation exposure
- *Seidenberg et al. 1986*
  - No significant adverse offspring effects; organogenesis exposure
- *Gray and Kavlock 1984*
  - No significant adverse offspring effects; organogenesis exposure

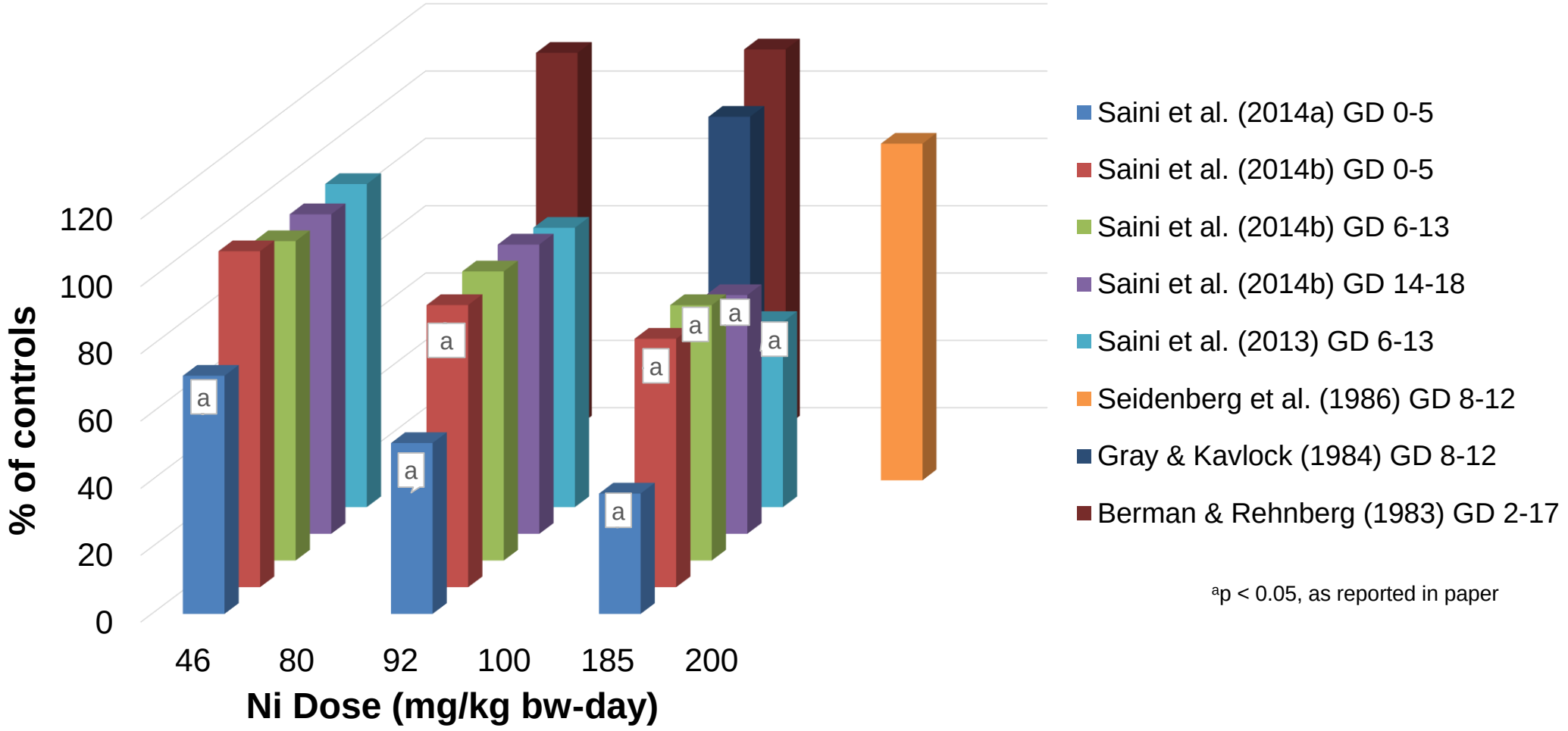
Oral Doses of Ni Tested in Rats and Mice



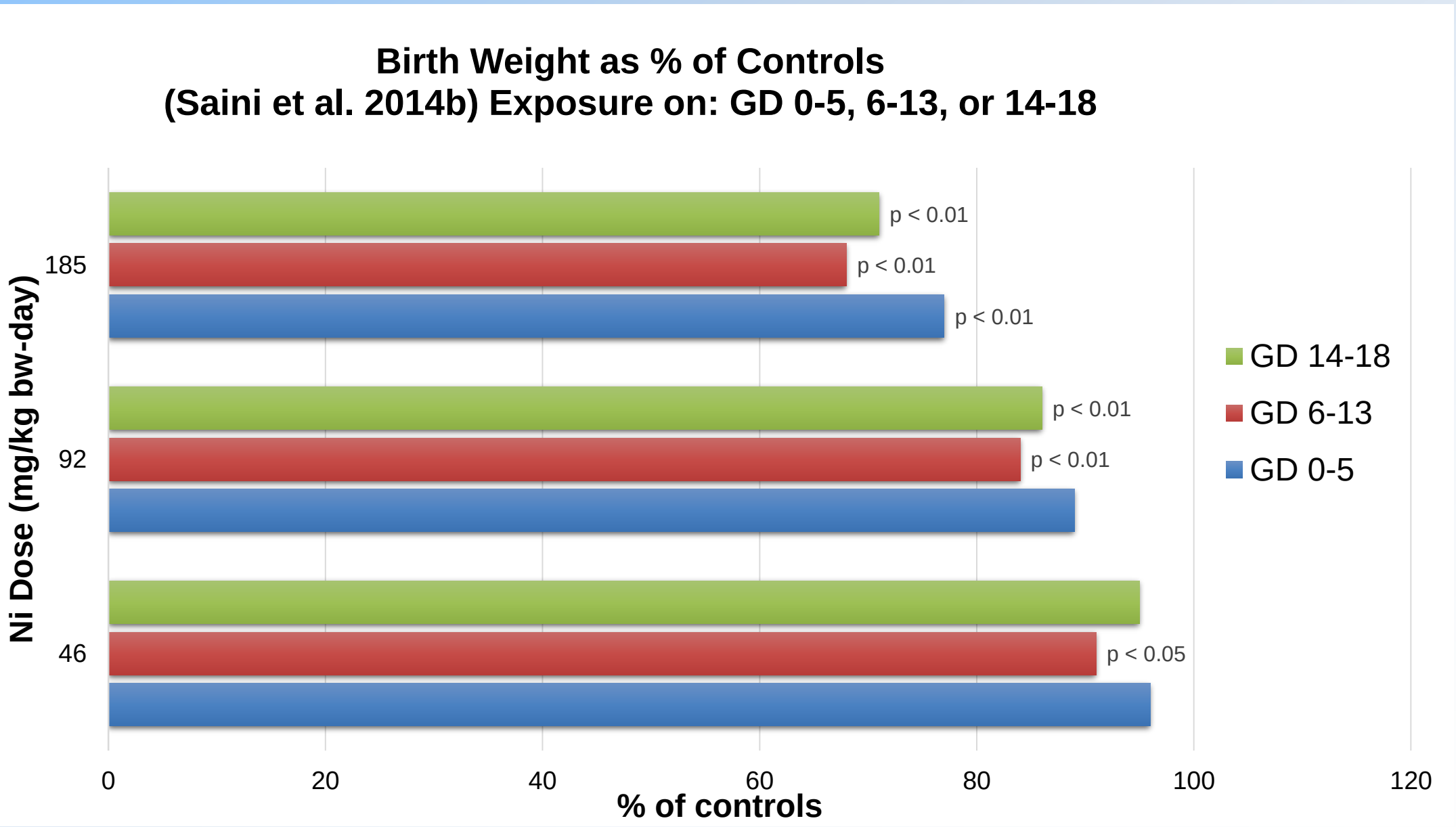

Live Litter Size at Birth as % of Controls
(Saini et al. 2014b) Exposure on: GD 0-5, 6-13, or 14-18
Ni Dose (mg/kg bw-day)
185
92
46
p < 0.05
p < 0.05
p < 0.05
P < 0.05
GD 14-18
GD 6-13
GD 0-5
0
20
40
60
80
100
120
% of controls

Oral Exposure in Mice: Live Litter Size as % of Controls

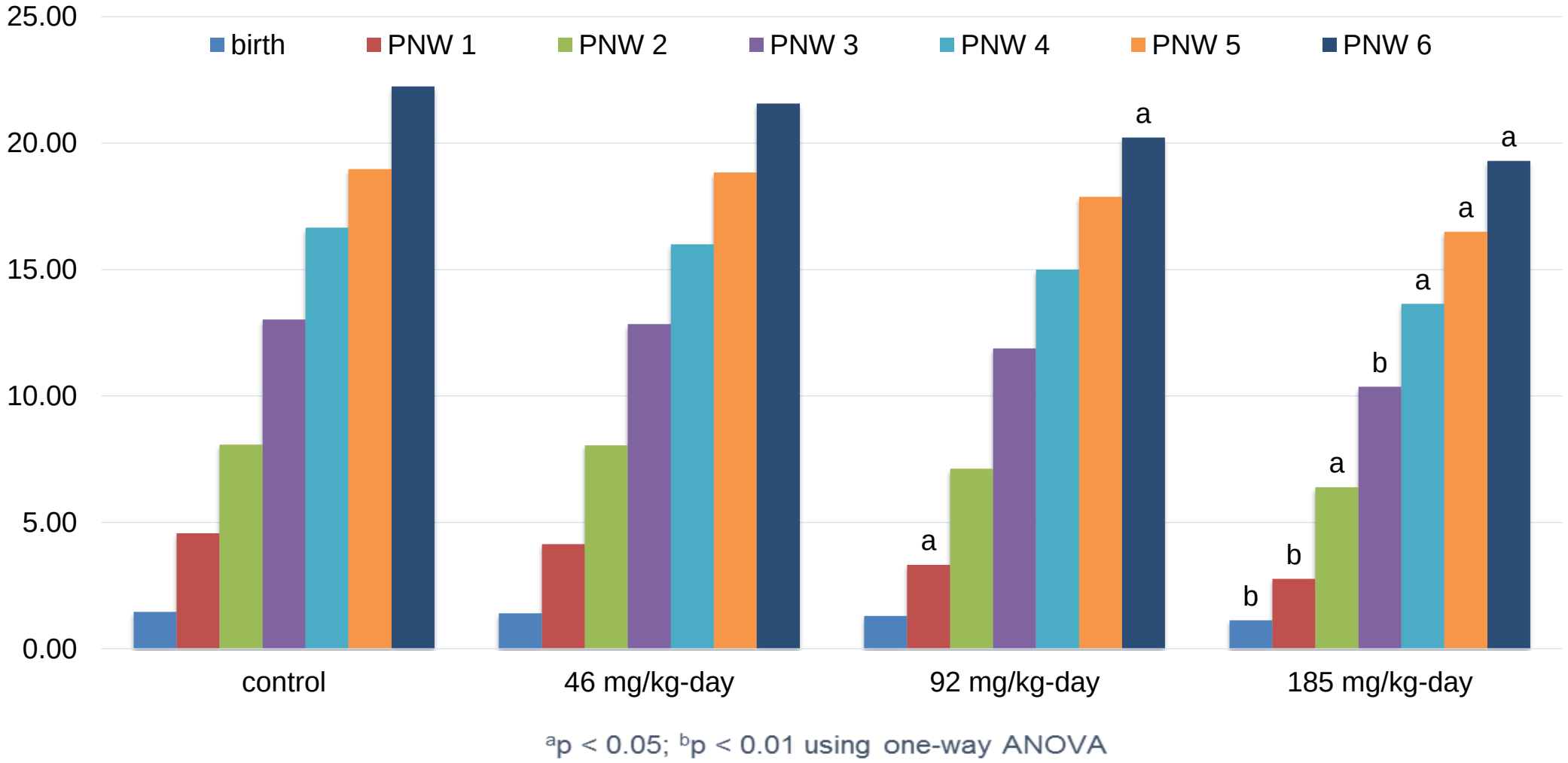
($NiCl_2$ for all studies)



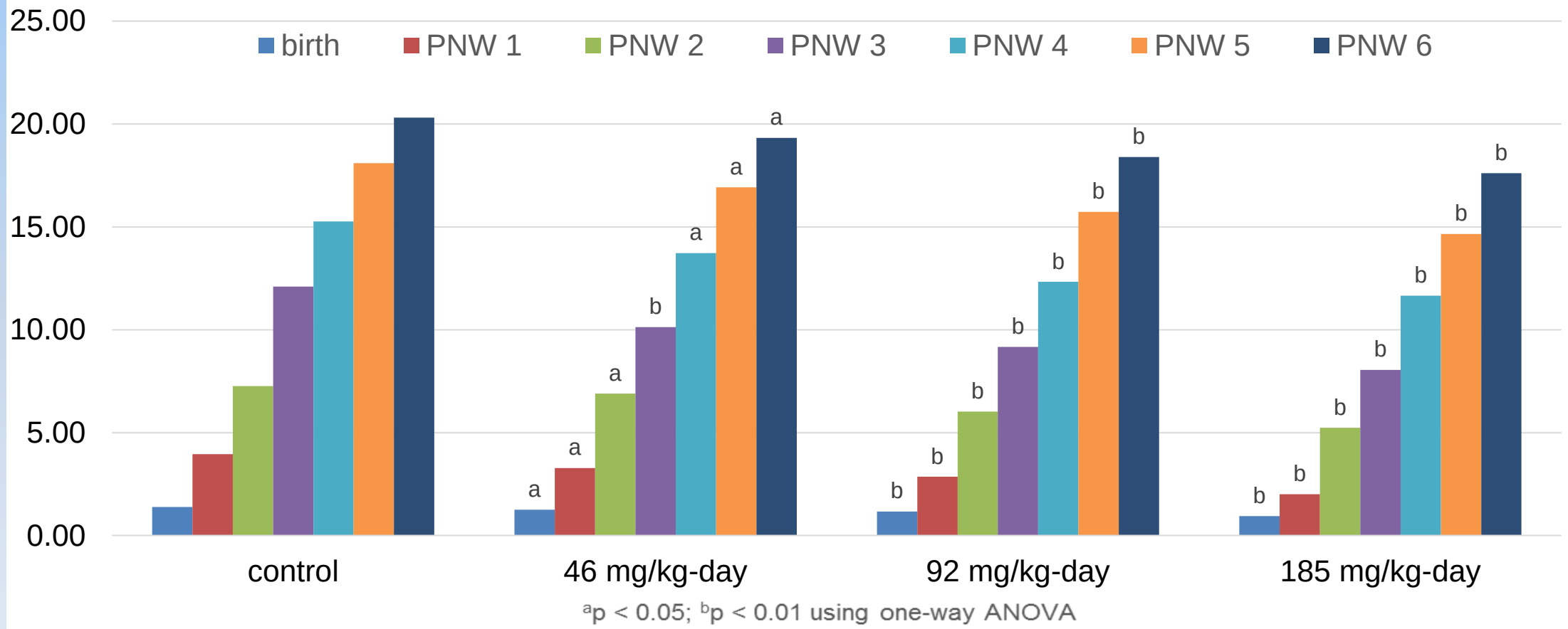
[a]$p < 0.05$, as reported in paper


Birth Weight as % of Controls
(Saini et al. 2014b) Exposure on: GD 0-5, 6-13, or 14-18
Ni Dose (mg/kg bw-day)
185
92
46
p < 0.01
p < 0.01
p < 0.01
p < 0.01
p < 0.01
p < 0.05
GD 14-18
GD 6-13
GD 0-5
0
20
40
60
80
100
120
% of controls

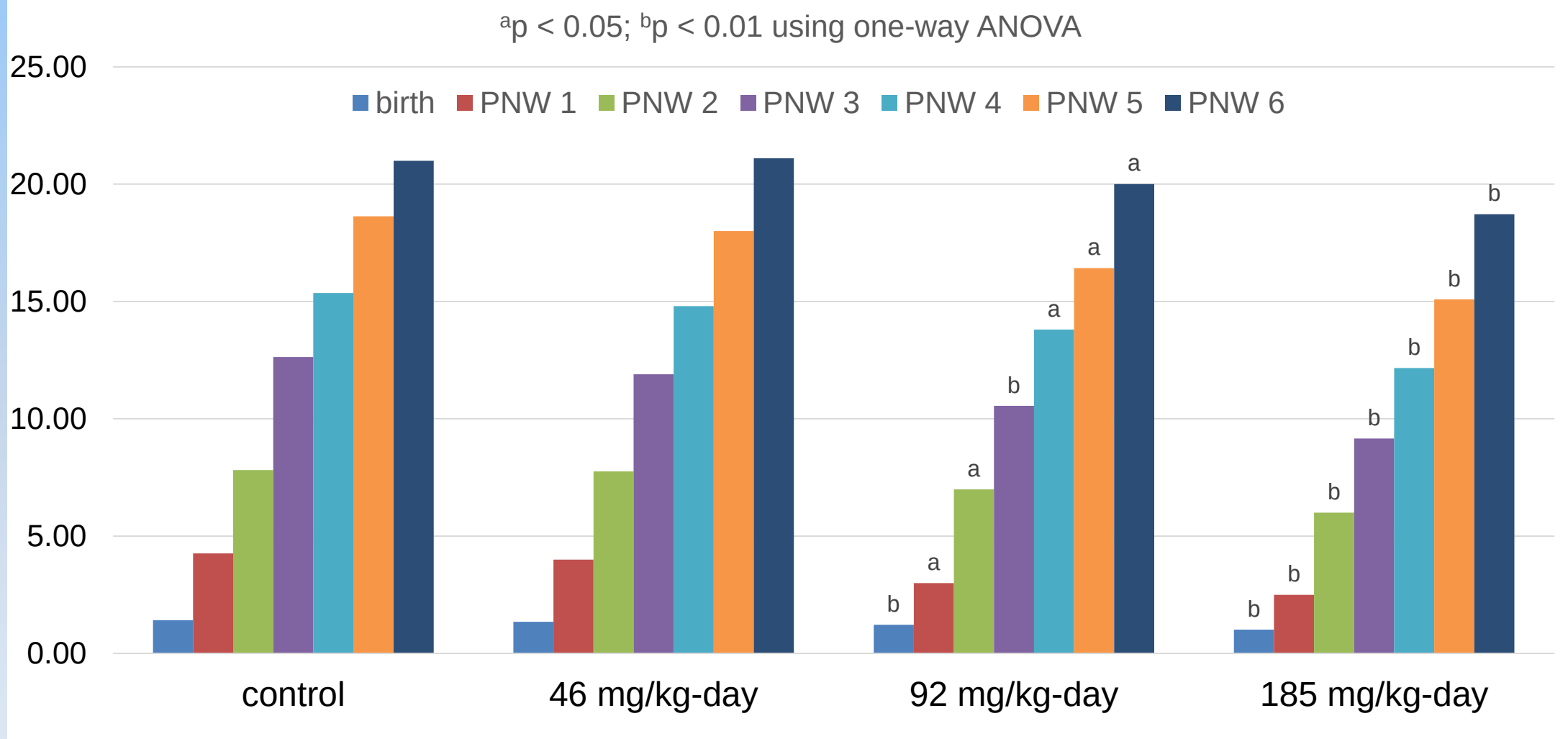
Saini et al. 2014b: Weekly Postnatal Growth (g) of Offspring Exposed to Ni During Preimplantation Period



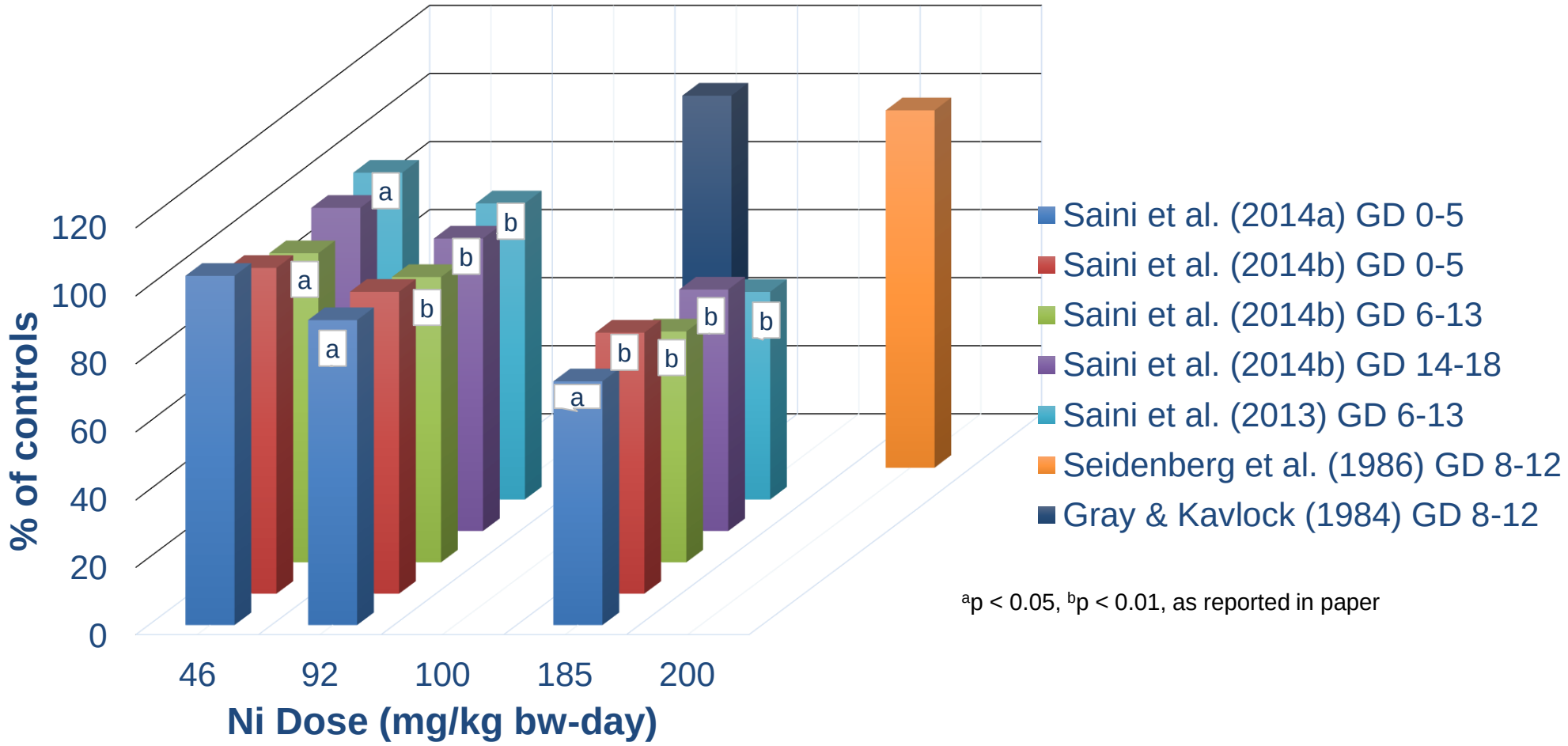
Saini et al. 2014b: Weekly Postnatal Growth (g) of Offspring Exposed to Ni During Organogenesis



Saini et al. 2014b: Weekly Postnatal Growth (g) of Offspring Exposed to Ni During the Fetal Period



Oral Exposure in Mice: Fetal/Birth Weight as % of Controls ($NiCl_2$ for all studies)



[a]p < 0.05, [b]p < 0.01, as reported in paper

Inhalation Exposure of Rats to Nickel Oxide (NiO)

Weischer et al. 1980

- Pregnant Wistar rats, 10/dose group and 13 air-exposed controls
- 0, 0.8, 1.6, or 3.2 mg/m^3
- Continuous exposure from mating through GD 21
- Decreased maternal gestational weight gain at all test concentrations ($p < 0.05$ at 0.8 mg/m^3)
- Decreased fetal weights at 1.6 and 3.2 mg/m^3 ($p < 0.01$)

Summary of Data on ip Injection Exposure to Nickel Compounds in Mice and Rats

*p < 0.05; ** p < 0.01; ^p < 0.005; ^^p < 0.001

Reference (species)	Compound, Method	Developmental Toxicity
Mas et al. 1985 (rats)	$NiCl_2$ ip	↓ fetal weight at 2* or 4* mg/kg given on GD 12 No effect on fetal viability
Chernoff and Kavlock, 1982 (mice)	$NiCl_2$ ip	↓ live litter size on PND 1* with 30 mg/kg on GD 8 No effect on pup weight on PND 1
Storeng and Jonsen, 1981 (mice)	$NiCl_2 \cdot 6H_2O$ ip	↑ of resorptions with 20 mg/kg given on any of GDs 1-5^ or 6** ↓ fetal weight with 20 mg/kg given on any of GDs 1-4 or 6^^, but not GD 5
Lu et al. 1979 (mice)	$NiCl_2$ ip	↑ frequency of fetal death; 100% with 6.9 mg/kg on GD 9, 10, or 11, and 5.7 mg/kg on GD 10 or 11 ↓ fetal weight seen at higher doses on all days, lowest effective dose/day 1.2 mg/kg on GD 10*

Summary of Data on im, sc, iv or intra-renal Injection Exposure to Nickel Compounds in Mice, Rats, and Hamsters

*p < 0.05; ** p < 0.01; ^p < 0.005; ^^p < 0.001

Reference (species)	Compound, Method	Developmental Toxicity
Sunderman et al. 1978 (rats)	$NiCl_2$ im (3 experiments)	↓ live litter size and postnatal growth with 16 mg/kg on GD 8** ↓ live liter size with 12** and 16* mg/kg on GD 8; ↓ fetal weight with 16 mg/kg on GD 8** ↓ live litter size with 3 or 4 mg/kg on GD 6-10*; no effect on fetal weight
	Ni_3S_2 im	↓ live litter size with 80 mg/kg on GD 6**; no effect on fetal weight
	$NiCl_2$ im	No effect on live litter size or fetal weight with 6, 8, or 16 mg/kg on GD 18 ↑ ratio of dead fetuses to total conceptuses^^ 50% maternal mortality with 16 mg/kg on GD 18
Adjroud, 2013 (rats)	$NiCl_2 \cdot 6H_2O$ sc	No effect on fetal weight with 25, 50, or 100 mg/kg on GD 3 ↓ live litter size with 100 mg/kg on GD 3**
Ferm, 1972 (hamsters)	"nickelous acetate"	↓ viability with 30 or 25 mg/kg on GD 8 No effect of 2 mg/kg
Sunderman et al. 1983 (rats)	Ni_3S_2 intra-renal	Single injection of 30 mg/kg 1 week prior to breeding associated with "intense" erythrocytosis in dams ↓ hematocrit in pups at 2 weeks postnatal age^^ ↓ pup weights at 2 ($p < 0.001$) and 4 weeks postnatal age, ^^for ♂; **for ♀

Summary of Human and Animal Developmental Toxicity

Human Data

- 5 cohort studies of air pollution reported small but statistically significant associations between Ni exposure and adverse effects on measures of fetal growth
- Results from studies of effects of Ni on ASD, spontaneous abortion, congenital defects, and preterm birth were inconsistent

Animal Data

- Regardless of species or route, the most sensitive and commonly reported adverse effects of prenatal exposure to nickel were reductions in viability and reductions in body weights of surviving offspring
- Both dose of Ni and timing of exposure were observed to impact the frequency of occurrence and the severity of effects

Human Female Reproductive Toxicity

Epidemiologic Studies of the Toxicity of Nickel and Nickel Compounds on the Female Reproductive System

Study	Exposure Assessment	Results
Bloom et al. 2011	Whole blood	No association with time to pregnancy
Zheng et al. 2015	Serum	1 µg/L increase in serum Ni was associated with adjusted 12.6% reduction in sex hormone binding globulin (p=0.03)
Maduray et al. 2017	Hair and serum	Ni in serum (p=0.16) and hair (p=0.85) were not significantly correlated with pre-eclampsia

Animal Female Reproductive Toxicity

Animal Studies of Female Reproductive Toxicity: Endpoints

- Uterus
- Ovary
  - Estrous cyclicity
- Reproductive index
- Milk composition & prolactin secretion

Uterus

Reference	Compound	Animal Model (Species)	Exposure	Doses/ Concentrations	Results
Rubanyi and Balogh 1982	$NiCl_2$	Pregnant Wistar rats	*In vitro* GD 20 uterine strips 1 hr incubation	10^{-7} – 10^{-3} M	10^{-7} M to 10^{-5} M $NiCl_2$ increased basal tone significantly 10^{-4} to 10^{-3} M $NiCl_2$ inhibited spontaneous contractile activity and decreased basal tone; dose-related mitochondrial structural damage and glycogen accumulation

Ovary

The effects of Ni vary from histological changes to functional alterations

Nickel has been reported to

- Disturb regular ovarian cycles ($NiSO_4$; Forgacs et al. 1997)
- Induce a dose-dependent anovulation ($NiSO_4$; Forgacs et al. 1997)
- Alter the secretion of several hormones, notably progesterone ($NiSO_4$; Forgacs et al. 1997, $NiCl_2$; Krockova et al. 2013)
- Cause histological alterations (Ni nanoparticles; Kong et al. 2014)
- Change weight and signs of oxidative stress ($NiCl_2$; Rao et al. 2009)

Estrous Cyclicity

Siglin (2000b). An oral (gavage) two-generation reproduction toxicity study in Sprague-Dawley rats with nickel sulfate hexahydrate.

- The mean cycle lengths of F0 females were 4.35, 4.36, 4.17, 4.63 and 4.48 days
- For F1 females, mean cycle lengths were 5.22, 5.66, 5.22, 5.47, and 6.04 days

- F1 females treated with increasing doses of Ni sulfate hexahydrate showed a trend toward significance for cycle lengths greater than 10 days (exact trend test had a p-value of 0.053)

	No. F1 females with one cycle length > 10 days / total females
control	**4/18 (22.2%)**
1.0 mg/kg-d	**10/24 (42%)** [p-value = 0.16]
2.5 mg/kg-d	**5/17 (29%)** [p-value = 0.46]
5.0 mg/kg-d	**7/16 (44%)** [p-value = 0.17]
10.0 mg/kg-d	**9/17 (53%) [p-value = 0.06]**

Reproductive index

Six studies were identified that examined endpoints which are encompassed by reproductive index

Measures of reproductive index are more clear when evaluated as a whole with consideration of developmental toxicity endpoints

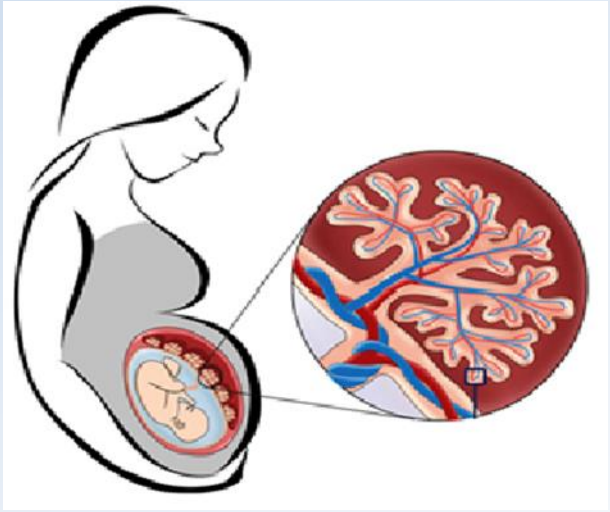
After oral administration or injection of $NiCl_2$ during pregnancy in mice and rats

- Increase in fetal death (Smith et al. 1993, Mizejewski et al. 1990)
- Reductions in body weights of fetuses and offspring (Saini et al. 2014)

Intraperitoneal injections of $NiCl_2$ in mice on the first day of gestation showed higher frequency of

- Both early and late resorptions (Storeng and Jonsen 1981)
- Stillborn and abnormal fetuses (Storeng and Jonsen 1981)

Maternal-fetal distribution of Ni



- Maternal-fetal transfer of Ni occurs in mammals via the placenta, and Ni has been detected in fetal blood and amniotic fluid
- Nickel has been detected in milk (Dostal et al. 1989)

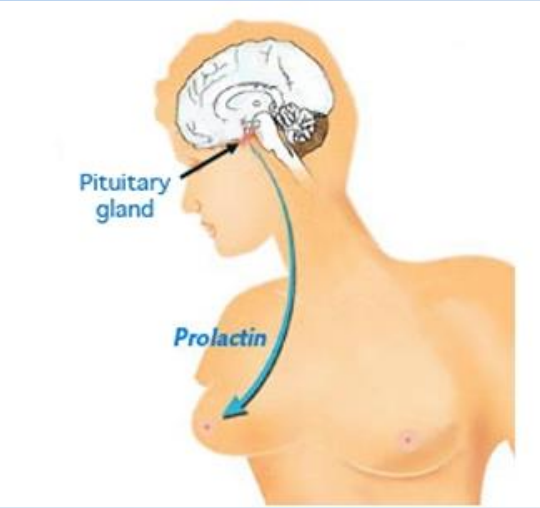
Milk composition

Three studies of Ni effects on milk composition were identified

- No significant effect on milk production, milk composition, animal health or feed consumption was seen in cows (O'Dell et al. 1970)

- Milk composition (solids, lipid, lactose, and fatty acid ratios) was reported to be altered in rats
  - Changes in milk quality and production was shown in rats after exposure to $NiCl_2$
    - ✓ Reductions in liver weight in the suckling pups (Dostal et al. 1989)
    - ✓ Rat pups consuming milk from Ni exposed mothers gained less weight (Kong et al. 2014)

Prolactin



- Prolactin secretion is reduced by exposure to $NiCl_2$ (LaBella et al. 1973; Clemons and Garcia 1981; Carlson 1984; Smith et al. 1993)

- Secretion of prolactin is a normal pituitary function

Summary of Female Reproductive Toxicity

Human Data

- Hormonal effects

Animal Data

- Estrous cyclicity, release of some hormones associated with reproductive function, and alterations to the uterus and ovary
- Neuroendocrine control of prolactin in rodents, and negative effects in offspring following changes in milk composition after the dams exposure to Ni and Ni compounds

Human Male Reproductive Toxicity

Epidemiologic Studies of the Toxicity of Nickel and Nickel Compounds on the Male Reproductive System

Exposure assessment

- Air
- Urine
- Semen
- Blood

Reproductive hormones

- Testosterone (T)
- T/luteinizing hormone (LH) ratio

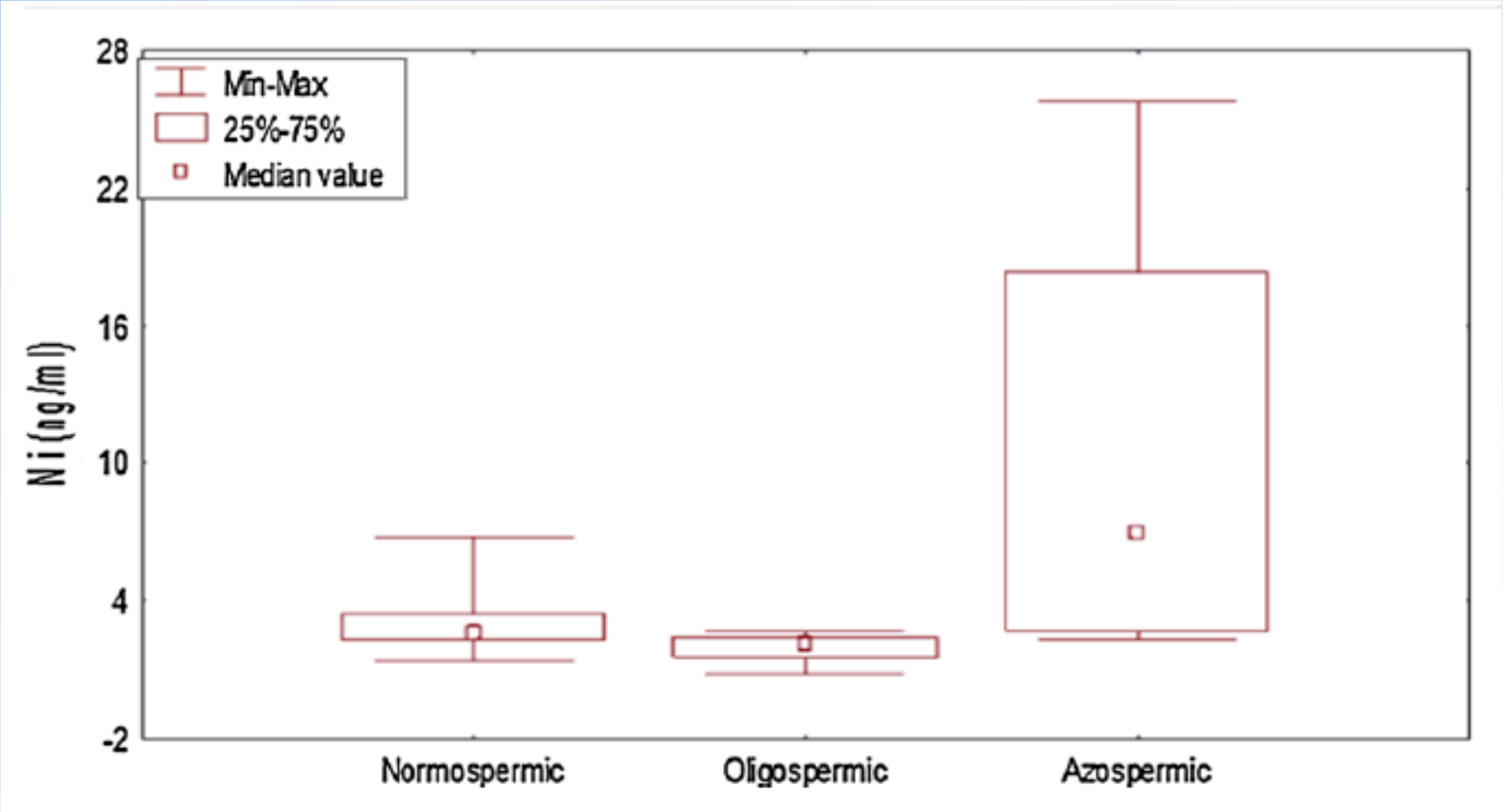
Sperm and semen parameters

- Morphology
- Concentration
- Volume
- Motility
- Viability
- DNA integrity
- Apoptosis

Semen Quality

Study	Exposure Assessment	Results
Danadevi et al. 2003	Blood	β=0.386 for Ni and % sperm with slow/nonlinear progressive motility, p=0.04
Slivkova et al. 2009	Semen	No associations
Skalnaya et al. 2015	Semen	↓ Semen volume < 1.5 mL, p=0.015
Zafar et al. 2015	Seminal plasma	↓ Sperm concentration ↓ Semen volume ↓ Sperm motility; all $p < 0.05$ Similar results for Cd $r > 0.70$ for Ni with Cd, Cu, Sn, and V

Semen Ni Concentrations in Normospermic (≥20 X 10^6 Sperm/ml), Oligospermic (<20 X 10^6 Sperm/ml), and Azoospermic (No Sperm) Subjects (Zafar et al. 2015)



Urine Ni and Sperm Quality

Study	Ni Exposure Assessment	Results
Zeng et al. 2015	Urine	β (CI) for % abnormal head and urine Ni concentration: 2nd quartile –1.65 (–3.9, 0.60) 3rd quartile 0.92 (–1.32, 3.16) 4th quartile 1.67 (–0.57, 3.92); p trend = 0.03
Zhou et al. 2016	Urine	Ni concentration in 4th vs. 1st quartile was associated with increase in comet tail length of 2.95 (0.34, 5.56) µm, adjusted for multiple metals

Urine Ni and Hormone Levels

Study	Exposure Assessment	Results
Zeng et al. 2013	Urine	β for testosterone and urinary Ni: 3rd quartile –83.79 (–163.85, –3.74) 4th quartile –36.35 (–116.31, 43.61) Ni was not retained in model with other metals
Sancini et al. 2014	Urine, air	β for log urinary Ni and log plasma testosterone: –0.466, p<0.001
Wang et al. 2016	Urine	The highest quartile of Ni was associated with 20% (38, 4) lower total T/LH ratio; 14% (32, 2) lower when adjusted for other metals.

Animal Male Reproductive Toxicity

Effects on Male Reproduction in Animal Studies

- Effects on sperm
- Histopathological effects - (testis, epididymis, seminal vesicle)
- Reproductive hormone changes
- Biochemical effects on the testis

$NiCl_2$, $NiSO_4$, Nickel nano and micro particles

Effects on Male Reproduction System
Effects on Sperm – Morphology, Motility & Mortality

- Dose-related ↑ abnormal sperm in seven species
  (rat, mouse, horse, ram, bull, boar, fox)
  Sperm abnormalities: head, neck and tail region

- ↓ Sperm count and motility in rats and mice:
  Seen with both normal and protein deficient diets
  protein deficient diet > normal diet

- Changes in motility of bovine sperm in the presence of Ni^{2+} *in vitro*

↓ Fertility index after male-only exposure in rats

Effects on Male Reproduction
Histopathological Effects

- **Testis -** Congestion and necrosis, ↑ frequency of localized apoptosis in the interstitium
  Seminiferous tubules – Degeneration, edema, or congestion in peripheral region, localized shrinkage, ↓ diameter, empty spaces in epithelium and cell death

- **Epididymis -** Degeneration, regressed epithelium of cauda, vacuolated cells
- *Action on the **epididymis** varies from that on the **testis***
- **Seminal vesicle –** Change of epithelium from high columnar to low cuboidal, indicative of lowered secretory activity

Effects on Male Reproduction
Reproductive Hormone Changes

- ↓ Serum testosterone, along with testicular damage in rats exposed to Ni nano and micro particles
- ↓ Serum FSH in rats exposed to Ni nano and micro particles

- Dose-related ↓ in stimulated testosterone production in the absence of cytotoxic effects in cultured mouse Leydig cells following either *in vivo* or *in vitro* Ni^{2+} treatment

- Reactive oxygen species (ROS) generation involved in ↓ testosterone production in cultured rat Leydig cells

Effects on Male Reproduction
Biochemical Effects in Testes

- ↑ Lipid peroxidation
- ↓ Antioxidant enzyme activities (e.g., SOD, catalase)
- ↓ GSH
- ↓ Serum and testicular L-ascorbic acid concentration and serum α-tocopherol levels
- ↑ Serum and testicular nitric oxide concentrations
- Oxidative stress associated with apoptotic cell death and DNA damage in testis and epididymal sperm
- Alterations in lactate dehydrogenase (LDH)
    - ↑ levels along with membrane integrity being affected
    - ↓ levels along with ↓ testicular protein

Summary of Effects on Male Reproduction

Human Data

Urinary Ni associated with

- Altered sperm morphology
- Lower plasma testosterone and T/LH ratio
- Sperm DNA damage

Animal Data

- Sperm: Alterations in morphology, motility and mortality
- Histopathology and biochemical effects
- ↓ Serum testosterone and FSH, ↓ testosterone production in cultured Leydig cells