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SEMEN QUALITY OF MEN APPLYING PESTICIDES IN NORTHWEST MINNESOTA

S.M. Schrader, T.W. Turner, P.B. Shaw, L.L. Erickson, S. Holland, and V.F. Garry. National Institute for Occupational Safety and Health, Cincinnati, Ohio and University of Minnesota, Minneapolis, MN

NIOSH and the University of Minnesota conducted a reproductive health study of men applying pesticides in northwest Minnesota. Two semen samples were collected from each of 90 study participants. The first semen sample was collected in July 1998 at the end of the herbicide application season and the second in late October 1998 at the end of the fungicide application season. A complete semen analysis was conducted on each semen sample including computerized semen analysis (HTM-IVOS, Hamilton-Thorn, Beverly, MA). The semen data were stratified by pesticide application history and analyzed for the effects of pesticides using generalized estimating equations (for discrete outcome variables) and the mixed model repeated measures approach (for continuous outcome variables). The percent normal sperm morphology (WHO) was significantly lower in the fungicide applicators (non-fungicide 29.8 % ± 2.6; fungicide 20.8% ± 3.4; $p = 0.016$). Fungicide applicators also had a lower sperm straight line velocity than the men not applying fungicides (non-fungicide 55.7 μm/sec ± 1.3; fungicide 48.4 μm ± 2.6; $p = 0.02$). A decrease in straightness of swimming path (VSL/VCL) was associated with both herbicide and fungicide application (non-herbicide 0.60 ± 0.03, herbicide 0.52 ± 0.01, $p = 0.02$; non-fungicide 0.58 ± 0.01, fungicide 0.54 ± 0.02, $p = 0.05$). The associations found in these analyses generate the need to separate the broad categories of herbicides and fungicides and study the association between specific pesticide chemicals and human semen quality. Further data analyses are underway detailing specific pesticide use based on current pesticide application records.

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DEVELOPMENT OF PIGS FOLLOWING *IN UTERO* AND LACTATIONAL EXPOSURE TO ORGANOCHLORINES: EFFECTS ON MALE REPRODUCTIVE FUNCTION.

B. Bérubé*, J.P. Laforest*, R. Sullivan, M.A. Sirard*, P. Ayotte* and J.L. Bailey, Centre de Recherche en Biologie de la Reproduction, Dépt des sciences animales, Université Laval, Québec, QC G1K 7P4.

The development and maintenance of reproductive tissues is to a large extent controlled by steroid hormones. Some environmental chemicals like organochlorines mimic, while others antagonize natural hormone activity when tested with *in vitro* assays or in whole animal models (Fry 1981; Jobling 1995). Typical organochlorine mixtures found in highly exposed human populations contain a large variety of organochlorine compounds, including substances with estrogenic, anti-estrogenic or anti-androgenic capacities (Moore 1997; Safe 1990). The objective of this study is to assess the impact of pre- and postnatal exposure to organochlorine mixtures found in the Arctic on the development and function of the male reproductive system, using the pig as the model. We present here the results of the first part of this study. Sixteen sows were randomly distributed to 4 treatment groups and administered various levels of a polychorinated biphenyl (PCB) cocktail composed of 15 organochlorine products (control; 1μg/kg PCB; 10 μg/kg PCB and 100 μg/kg PCB) from 4 months of age until their first litter (~34 weeks). Treatment had no effect on the weight gain of the sows (269 ± 5 kg) and no anatomical or fertility effects were observed. The PCB concentration in the serum of the 100 μg/ml group was 14.4 ± 0.6 μg/L after 30 weeks of treatment. Prepubertal piglet development appeared unaffected by the treatments. Semen analyses will be performed on the pigs as reach puberty. At this time of the experiment, we cannot conclude that this organochlorine mixture affects the growth rate and fonctional development of the reproductive system of the pig. *This study is supported by the Arctic Environmental Strategy Northen Contaminant Program Branch of Health Canada.*

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THE CONTROL OF SPERMIATION IN THE RAT

R.E. Chapin, M.W. Harris*, J.K. Haseman* and R.N. Wine*, Reproductive Toxicology Group and Biostatistics Group, NIEHS/NTP, RTP, NC

Because most testicular toxicants inhibit spermiation during the pathogenesis of a testicular lesion and little is known about this process, we have developed an *in vitro* model to explore the control of late spermatid release. When incubated overnight, short segments of stage VII-VIII tubules from adult SD rats evert at both ends, exposing the epithelium to the medium. With gentle rocking, progression can be observed histologically (spermiation, elongation of round spermatids, basal movement of residual bodies), and sperm in the medium can be counted to quantify release. Although not all sperm are released in control tubules, this provides an initial model with which to begin the study of this process. For control tubules in DME/F12 medium with ITS+®, EGF, and Testosterone, the degree of release was found to be highest in stage VIII tubules (50-60%), and lowest in stage VI tubule fragments (14%), while stage VII tubules gave intermediate values (≈27%). Sodium azide (1.3 – 2.6 mM) and sodium cyanide (1.7 mM) inhibited release by ≈ 40%. The omission of EGF from the medium, or the inclusion of the EGF kinase blocker PD168393 (30 nM), both lowered release, suggesting the involvement of EGF and protein phosphorylation. Indeed, previous immunohistology suggested that protein phosphorylation might play a key role in spermiation. In support of this hypothesis, the phosphatase inhibitors okadaic acid (30-100 nM) and peroxovanadate (1.25 -3.8 μM) produced dose-related increases in sperm release. This model provides a tool for the study of both the hormonal control of spermiation, and mechanisms of toxicant-induced disruptions. The data gathered to date are consistent with the concept that protein phosphorylation is involved in controlling sperm release.

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EFFECT OF A MIXTURE OF ENVIRONMENTAL CONTAMINANTS ON THE MALE RAT

E.V.Younglai, W.G.Foster*, M.G.Wade* and C.L.Hughes*, Department of Obstetrics & Gynecology, McMaster University, Health Sciences Center, Hamilton, Ontario, Canada, L8N 3Z5.

Our current understanding of the toxicity of persistent chemical pollutants to the human population is derived, to a large extent, from studies in rodents exposed to high doses of a single toxic agent. Little is known about their impact at low concentrations, such as exist in the environment. We assessed the reproductive toxicity of a complex mixture of organochlorine pollutants in the adult male rat. The mixture contained persistent organochlorine pesticides and industrial chemicals to which the general population in North America are chronically exposed. It included dioxin, PCBs, DDT metabolites, dieldrin, methoxychlor, mirex, polychlorinated benzenes, lead and cadmium.

Adult male rats were gavaged with the mixture daily for 72 days at doses equivalent to 1X, 10X, 100X and 1000X the MRL (minimum risk level) for each component. Liver and kidney weights were elevated at the highest dose. Adrenal, thymus, reproductive organs, sperm production or circulating and pituitary levels of LH, FSH, prolactin and testosterone were not affected. The T4/TSH ratio was suppressed three-fold. Hepatic microsomal UDP glucuronyl transferase (1000X MRL), EROD (10X) and BROD (100X) were significantly elevated. Liver from the 1000X treatment group had many lesions. Serum lactate dehydrogenase and urea nitrogen were depressed 50% at 100X MRL. In summary, low dose exposure of a mixture of persistent pollutants had little effect on male rat reproductive processes but at high levels had adverse impacts on thyroid hormone levels, and liver and kidney. *(Supported by the Canadian Chlorine Co-ordinating Committee)*