SUMMARY REPORT:

HUMAN REPRODUCTIVE ENDOCRINE EFFECTS OF CONTROLLED TOLUENE EXPOSURE

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ABSTRACT

Despite previous observations of adverse reproductive effects of toluene, including alterations of serum gonadotropins (luteinizing hormone, LH, and follicle-stimulating hormone, FSH) in men, little is known regarding the mechanism of toxicity. We tested the hypothesis that toluene acutely suppresses pulsatile gonadotropin secretion by measuring LH and FSH at frequent intervals during controlled exposure to toluene.

Women in the follicular and luteal phases of the menstrual cycle and men were randomized to inhale filtered air with or without 50 ppm toluene by mouthpiece for 3 hours (19% of the OSHA Permissable Exposure Limit). Blood sampling by intravenous catheter was done at 20 min intervals for 3 h before, 3 h during and 3 h after exposure. Plasma LH, FSH, and testosterone were measured. LH and FSH pulse amplitude, pulse frequency, and mean concentrations for each of the 3 h periods before, during and after toluene versus sham exposure were calculated using the ULTRA pulse detection program and compared by analysis of variance with repeated measures.

In men a significant interaction ($p<0.05$) between exposure and sampling period on LH mean levels was observed, with a greater LH decline during toluene than sham exposure. However, there was no concomitant effect on testosterone levels. In luteal phase women a trend towards a statistically significant interaction between exposure and sampling period ($p=0.06$) on LH pulse frequency occurred, with a greater decline in pulse frequency during toluene than sham exposure. There were no other significant effects of toluene exposure.

Three hour exposure to 50 ppm toluene did not result in abnormal episodic LH or FSH secretion profiles, however, subtle effects on LH secretion in men and luteal phase women were observed. The clinical significance of the observed effects is unclear, underscoring the need for further study of reproductive function in exposed workers.

SIGNIFICANT FINDINGS

In follicular phase women there was no significant effect of toluene exposure alone, nor were there significant interactions between exposure and sampling period, on LH mean concentration, pulse amplitude, or pulse frequency or on FSH mean or log FSH mean concentrations. There was a significant effect of sampling period alone ($p=0.04$) on LH mean concentration and log LH mean concentration, in the form of slightly increasing mean LH levels in both sham- and toluene-exposed women over the 3 sampling periods. Very few significant FSH pulses were observed in the toluene-exposed group and no FSH pulses were observed in the sham-exposed group. Therefore, analyses were not done for FSH pulse amplitude or pulse frequency in follicular phase women.

In luteal phase women there was no significant effect of toluene exposure, nor were there significant interactions between exposure and sampling period, on LH mean levels, pulse amplitude, or pulse frequency. However, there was a trend towards a significant interaction ($p=0.06$) between sampling period and exposure on LH pulse frequency, with pulse frequency apparently reduced during the exposure period in the exposed compared to the sham-exposed women. There was a significant effect of

sampling period alone on LH mean levels ($p=0.02$) and pulse frequency ($p=0.04$), reflecting the occurrence of most of the luteal phase pulses during the pre- and post-exposure intervals in both sham- and toluene-treated groups. There was no significant effect of treatment, nor was there interaction between treatment and sampling period on mean FSH levels; however, there was a borderline significant effect of sampling period alone ($p=0.05$), reflecting slightly higher mean FSH levels pre-exposure compared to during and after exposure in both sham and treated groups. There were no significant effects on FSH pulse amplitude or frequency.

In the men there was no significant effect of treatment alone on any parameter of LH secretion, however, there was a significant interaction ($p<0.05$) between treatment and sampling period on LH mean concentration. This appeared in the form of slightly declining mean LH levels over the exposure intervals in the toluene exposed individuals, while LH levels decreased and then increased slightly during and after exposure in the sham-exposed individuals. There were no significant effects of treatment or exposure interval on FSH mean concentration, log FSH mean concentration, pulse amplitude, or pulse frequency in men.

Plasma testosterone concentrations measured at the end of each of the three time intervals in the men did not differ significantly by exposure or sampling period.

Mean peak whole blood toluene levels in both toluene-exposed and sham-exposed subjects measured at the end of the 3 h exposure period did not differ by gender or menstrual cycle stage.

USEFULNESS OF FINDINGS

The study was designed to test the hypothesis that exposure to toluene at 50 ppm for 3 hours acutely suppresses LH and FSH secretion via suppression of hypothalamic GnRH pulse frequency in men and women. This exposure is equivalent to 38% of the ACGIH TLV-TWA, 38% of the British regulatory exposure limit, and 19% of the United States and German regulatory exposure limits (ACGIH 1991). Exposure to toluene at this level did not result in abnormal LH or FSH pulse amplitude, pulse frequency or mean levels in either men or women, as defined by previous studies of spontaneous episodic LH and FSH secretion in young men (Matsumoto and Bremner 1984; Spratt et al. 1988) and in luteal and follicular phase women (Soules et al. 1984; Soules et al. 1985; Filicori et al. 1986; Rossmanith et al. 1990). Since each peripheral LH pulse is a reflection of a hypothalamic GnRH pulse (Plant 1986), the absence of significant effects of toluene exposure on LH pulse frequency also suggests that acute toluene exposure at this level likely does not alter GnRH pulse frequency. Of interest, however, was 1) a trend towards a significant decline in LH pulse frequency in luteal phase women during toluene exposure ($p=0.06$), and 2) a small, but statistically significant, decline in LH mean levels in toluene-exposed men compared with the sham group.

The clinical significance of the observed effects is unclear. The mean LH levels in all of the sham and toluene-exposed men remained within the normal ranges for LH

mean concentrations which were established by the laboratory which performed the assays (Personal Communication). Therefore, the observed effect on mean LH levels may represent a subclinical effect of toluene on the pituitary or hypothalamus without impact on testicular testosterone secretion. The trend towards decreased LH pulse frequency in luteal phase women during toluene exposure is also difficult to interpret. It may be coincidental since LH pulse amplitude and mean concentration also declined during sham-exposure, although not to the same degree as during toluene exposure. Although the very low LH pulse frequency observed in the toluene-exposed women during the 3 hour exposure period falls outside of the normal range of about 0.2 to 0.5 pulses/hour during a 24 h period in the mid-luteal phase, periods of 4 to 5 hours in duration with no significant LH pulses occur commonly in women during this phase (Soules et al. 1984; Soules et al. 1985; Filicori et al. 1986; Rossmanith et al. 1990). For comparison LH pulse frequencies of 0 to 0.25 pulses/hour have been reported in women with hypothalamic amenorrhea or oligomenorrhea (Veldhuis et al. 1985; Southworth et al. 1991). Because of the low LH pulse frequency in normal luteal phase women, longer durations of exposure with frequent blood sampling may be necessary to determine whether toluene exposure suppresses LH pulse frequency in luteal phase women. This would not be feasible with our exposure system because of discomfort to the subjects caused by prolonged sitting while breathing through a mouthpiece. It would be possible, however, to perform longer controlled exposures using an exposure chamber.

This study cannot rule out the possibility that exposures to toluene which result in higher blood toluene concentrations or in comparable toluene concentrations for longer durations may have greater effects on gonadotropin secretion in men and women than we observed. As discussed above, longer exposures by mouthpiece are impractical. However, exposures to higher toluene concentrations within recommended exposure limits could help to establish whether currently allowable exposures have significant effects on reproductive endocrine function. Although further controlled exposure studies can help to clarify the effects of acute toluene exposures on reproductive hormones, they cannot be used to study the effects of chronic exosures. Our findings therefore underscore the need for further epidemiological studies of reproductive function in workers exposed chronically to toluene to establish the safety of present occupational exposure limits. The companion technical report to this summary report discusses the results of an epidemiological study of reproductive endocrine function and fecundability in solvent-exposed workers.

PUBLICATIONS

Luderer U, Morgan MS, Brodkin, CA, Kalman DA, Faustman EM: Reproductive endocrine effects of acute toluene exposure in men and women. Occupational and Environmental Medicine 56: 657-666, 1999.

Luderer U, Kalman DA, Morgan MS, Faustman EM: Effects of controlled toluene exposure on gonadotropin secretion in men and women. Toxicol Lett 95(Suppl 1):214, 1998.

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The experiments described in the above publication and abstract addressed the Specific Aims of the project as follows:

Specific Aim 1. To assess whether exposure to levels of toluene under the currently allowable limits alters gonadotropin (luteinizing hormone and follicle stimulating hormone) secretion in humans.

LH and FSH were measured in blood samples drawn before, during, and after controlled exposure to toluene for 3 h in men and women. The exposure level of 50 ppm for 3 hours is equivalent to 19% of the Occupational Safety and Health Administration Permissable Exposure Limit of 100 ppm 8 h time-weighted average and 37.5% of the American Conference of Government Industrial Hygienists Threshold Limit Value of 50 ppm. The importance of the possible effects observed on LH secretion in men and in luteal phase women are discussed below.

Specific Aim 2. To explore whether the differences in toluene pharmacokinetics and hormonal milieux between men and women affect their gonadotropin responses to toluene exposure.

Gonadotropin responses to acute toluene exposure were investigated in three groups of subjects with very different hormonal milieux: men, women in the follicular phase of the menstrual cycle, and women in the luteal phase of the menstrual cycle. While we observed no effects on gonadotropin responses in follicular phase women, the data suggested that LH secretion in men and luteal phase women might be slightly affected by toluene exposure. This raises the possibility that high progesterone levels (in the luteal phase women) or testosterone levels (in the men) might increase the sensitivity of the hypothalamic-pituitary axis to toluene. However, the results were not consistent in that the other parameters of pulsatile LH secretion were not affected by the exposure. For example in the men, one would have more confidence in the effect on mean LH concentration if LH pulse amplitude and/or pulse frequency had similarly been affected or if a concomitant effect on testosterone secretion had been observed.

Pharmacokinetic studies of controlled toluene exposures in men and women by our collaborators demonstrated that most of the difference between men and women in toluene elimination is due to the greater body fat percentage of women (Mar et al. 1998). As adiposity also affects gonadotropin secretion, we examined the effects of including two different indicators of adiposity, body fat percentage and body mass index, on our statistical model of the effects of toluene exposure on LH and FSH secretion. Neither of these covariates was found to significantly alter the models.

Specific Aim 3. To explore how gonadal feedback modulates the effects of toluene exposure on gonadotropin secretion in women.

As discussed above, we observed no effects of toluene exposure on gonadotropin secretion in follicular phase women, but observed a non-significant tendency towards decreased LH pulse frequency with toluene exposure compared to sham-exposure in the luteal phase women. The importance of this non-significant trend was considered

questionable because significant changes in LH mean levels and pulse amplitude were observed with **both** toluene- and sham-exposure.

We did not conduct the second experiment under this Specific Aim (controlled toluene exposures in oophorectimized women) because of the absence of significant effects in non-oophorectimized women.

REFERENCES

ACGIH (1991). Documentation of the Threshold Limit Values and Biological Exposure Indices.

Filicori, M, Santoro, N, Merriam, G R, Crowley, J, W.F. (1986). "Characterization of the Physiological Pattern of Episodic Gonadotropin Secretion thoughout the Human Menstrual Cycle." J Clin Endocrinol Metab **62**: 1136-1144.

Mar, T F, Pierce, C H, Morgan, M S, Dills, R S, Shen, D D, Kalman, D A (1998). "The Effects of Exercise and Gender on Toluene Toxicokinetics in Human Volunteers [abstract]." Toxicol Sci **42 (Suppl1)**: 142.

Matsumoto, A M, Bremner, W J (1984). "Modulation of Pulsatile Gonadotropin Secretion by Testosterone in Man." J Clin Endocrinol Metab **58**(4): 609-614.

Plant, T M (1986). "Gonadal Regulation of Hypothalamic Gonadotropin-Releasing Hormone Release in Primates." Endocrine Rev **7**(1): 75-88.

Rossmanith, W G, Liu, C H, Laughlin, G A, Mortola, J F, Suh, B Y, Yen, S S C (1990). "Relative Changes in LH Pulsatility during the Menstrual Cycle: Using Data from Hypogonadal Women as a Reference Point." Clin Endocrinol **32**: 647-660.

Soules, M R, Steiner, R A, Clifton, D K, Cohen, N L, Aksel, S, Bremner, W J (1984). "Progesterone Modulation of Pulsatile Luteinizing Hormone Secretion in Normal Women." J Clin Endocrinol Metab **58**(2): 378-383.

Soules, M R, Steiner, R A, Cohen, N L, Bremner, W J, Clifton, D K (1985). "Nocturnal Slowing of Pulsatile Luteinizing Hormone Secretion in Women during the Follicular Phase of the Menstrual Cycle." J Clin Endocrinol Metab **61**(1): 43-49.

Southworth, M B, Matsumoto, A M, Gross, K M, Soules, M R, Bremenr, W J (1991). "The Importance of Signal Pattern in the Transmission of Endocrine Information: Pituitary Gonadotropin Responses to Continuous and Pulsatile Gonadotropin-Releasing Hormone." J Clin Endocrinol Metab **72**: 1286-1289.

Spratt, D L, O'Dea, L S L, Schoenfeld, D, Butler, J, Rao, P N, Crowley, J, W.F.. (1988). "Neuroendocrine-Gonadal Axis in men: Frequent Sampling of LH, FSH, and Testosterone." Am J Physiol **254(Endocrinol. Metab)**: E658-E666.

Veldhuis, J D, Evans, W S, Demers, L M, Thorner, M O, Wakat, D, Rogol, A D (1985). "Altered Neuroendocrine Regulation of Gonadotropin Secretion in Women Distance Runners." J Clin Endocrinol Metab **61**(2): 557-563.