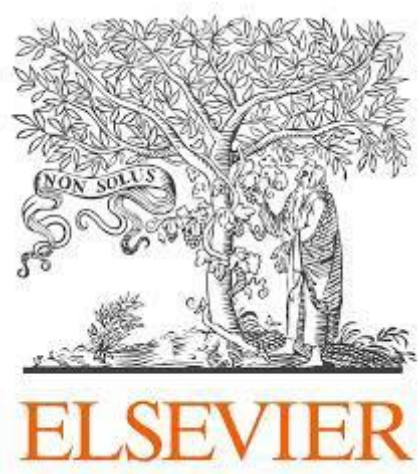
MEDICAL TEACHER



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The evaluations of exposure to Laprol-604 during pregnancy in wistar rats

Abstract: The high production volumes and widespread use of surfactants have been an environmental concern since the early 1960s. Surfactants are used in industry and agriculture and are also found in household and personal care products. The presence of surfactants and their biodegradation products in different environmental compartments can invoke a negative effect on the biota [11]. The toxicity of surfactants to living organisms has been summarized in the scientific literature. Nevertheless, some information is still lacking in relation to the reproductive effects of surfactants and interactions with hormonal system, also need to be understood in order to avoid unexpected adverse effects on future generations of people and the environment.

Pregnant Wistar rats were administered 0,125; 1,25 and 12,5 mg/kg Laprol-604 once daily by gavage from the second gestation day (GD) to the twenty first GD. Controls consisted of 20 intact pregnant Wistar rats without Laprol-604 administration. Time of parturition for each animal, number of live pups and their conditions have been examined. All of live pups were daily counted, tabulated and they have been weighed several times during postnatal period. All live pups have been born by rats exposed to Laprol-604 12,5 mg/kg were pallid, inactive and died within the first 48 hours after birth. Approximately 50% of the pups of pregnant rats administrated 1,25 mg/kg died during the first 10 days after birth. Other 50% of these animals survived and reached puberty, but they had significant growth retardation. The surviving young rats had significantly higher liver weight compared to the control animals.

Survival has been improved by lower dosage Laprol-604 administration and over 80% pups exposed to 0,125 mg/kg Laprol-604 have been born alive, stayed active for postnatal life and reached puberty. The results of study indicated that

exposure of Laprol-604 to the pregnant rats caused decreasing postnatal survival of neonates, stunted growth in the surviving rat pups.

Keywords: Laprol-604, polyols, surfactant, modeling, rats, reproductive toxicity, developmental toxicity, gestation day, postnatal day.

Introduction. Population exposure to toxic environmental chemicals is ubiquitous and adverse health outcomes associated with exposure to such chemicals as surfactants are prevalent and on the rise [15, 20]. Surfactants have widely used all over world. A large number of surfactants containing wastewater are discharged into the environment, resulting in harming aquatic life, polluting the water and endangering human health [10, 14, 21].

Wildlife and humans are exposed to surfactants in several different ways. Air, water, soil, sediment and food are sources of polyols for living organisms. With regard of lack of toxicokinetic data, it is known that women may be exposed to lipophilic chemicals from various sources including air, water, food, occupational and household environments. Lipophilic chemicals can be stored and accumulated over time in body fat, have the capacity to pass through the placental barrier and into the fetal blood stream. Surfactants can also be transferred from the pregnant woman to the developing fetus through the placenta. The most sensitive time of exposure to surfactants during critical periods of development, such as during fetal development.

Laprol-604 is found among non-ionic group of surfactants, including Laproxide-303, methycellosolve, methylcarbitol et cetera [22]. Laprol-604 has been produced industrially for several decades for use primarily as ingredients of manufacturing epoxide resin, enamels, varnishes, plastic, fiber, glues, emulsifiers et cetera [22]. Laprol-604 made through the use of its exhibit valuable commercial properties that include water and oil solubility, enough stability and thus found extensive utilization.

The potentially toxic effects of surfactants are presently being studied with increasing intensity. The relevance of this topic is also clearly reflected by the number of publications that have appeared in recent years [1, 2, 16]. This increasing interest is the result of reports of toxic effects of different groups of surfactants in connection with the ubiquitous detection of these substances in the environment and in sundry matrices, bodies of water, wild animals, human blood, and breast milk samples, all of which have come to the attention of the public [8, 9, 12].

Nonionic surfactants have been most intensively studied from a toxicological standpoint [16, 22]. Multiple reports, in a variety of animal models, paint a concerning picture of the potential for surfactants to contribute to negative health effects, including developmental toxicity [7], immunological suppression [3], in neonatal mortality. Investigations show that biological action of non-ionic surfactants are characterized by numerous structural metabolic disorders, as well as possibility to condition remote consequences, such as acceleration of body aging, immune deficiency, inhibition of generative function, atherogenesis, mutagenesis and so on [18, 19, 22]. Although the toxicity of Laprol-604 has been studied and the findings showed that Laprol-604 was moderately toxic, however the influence of Laprol-604 on reproductive system is still unknown.

Survival of the newborn rats, their body weight, liver status as well as their thyroid status have been examined with the goal of researching the reproductive and developmental toxicities of Laprol-604.

Materials and methods. Laprol-604 was provided from Science and Production Joint Stock Company "Sintez PAV" (Shebekino, Russian). Laprol-604 was reported to be 96% pure by the supplier. For all studies, Laprol-604 was diluted in deionized water and prepared fresh daily. According to biologic characteristic of Wistar rat, the placenta is considerably more porous. This property may increase the chance of fetal exposure to an administered test material

One hundred pregnant Wistar rats (body weight, 180±30 g at study start) bred within a 4-h period in the afternoon and overnight. Those animals with spermatozoa in a vaginal smear were considered to be at gestation day (GD) 0. They were randomly divided into four groups (25 animals in each group). Laprol-604 was administered to pregnant dams once daily by gavage at doses of 0,125; 1,25 and 12,5 mg/kg, respectively is the 1-st; 2-nd and 3-rd group from GD 2 until GD 19. The 4-th group (controls) consisted of 25 intact animals without Laprol-604 administration. The pregnant rats were kept individually in polypropylene cages with heat-treated pine shavings for bedding and tap water ad libitum. Pelleted diets were presented to the rats in wide mouthed jars with lids. Animal facilities were controlled for temperature (20-22°C) and relative humidity (50-60%) and kept under a 12-hr light/ 12-hr dark cycle. All the procedures were performed at Kharkiv Medical Academy of Postgraduate Education, according to Ukrainian and International guidelines for the use of animals in research [5, 6].

Pregnant rats routinely monitored during study as an assessment of their general health and to effect of Laprol-604 administration. Daily observations were performed first thing in the morning and last thing before leaving in the afternoon to assess the health of the dams. In this observation, behavioral status, respiratory signs, skin, eyes, and excretory products were noted. Pregnant rats have been weighed on the 0, 6, 9, 12, 15, 18, 20-th days of gestation. Throughout rat gestation food and water consumption were recorded. On 20-th gestational day pregnant rats were euthanized for humane reasons to prevent autolysis and tissue loss. During the necropsy, an external examination of the pregnant rats was done, all organs were examined, in situ; the number of yellow bodies were counted in the ovaries. The gravid uteruses were removed, examined and weighed. The numbers and positions of the live or dead fetuses, as well as resorptions, were recorded. Live fetuses were weighed individually, gender-determined, and examined for external abnormalities, craniocaudal dimensions were determined. Half of the fetuses were prepared for skeletal examination. They were killed with an overdose of pentobarbital, eviscerated, and fixed in 95% ethanol. Specimens were subsequently stained with Alizarin red and Alcian blue to visualize bone and cartilage, respectively. Skeletal morphology was evaluated, according to "Examination of the axial skeleton of fetal rodents" [17]. The other half of fetuses were prepared for visceral evaluation. They were fixed in Bodian's solution (2% formaldehyde, 5% acetic acid, 72% ethanol, 21% water). Examination of the head, thoracic, and abdominal viscera were carried out using a freehand razor dissection.

Statistical analysis of the data was performed using GraphPad Prism 5. Student's t test was used to detect differences between independent groups of normally distributed variables; difference between groups was considered statistically significant at $p<0.05$.

Results. Impacts of Laprol-604 on weight gain in pregnant rats were 25,10±1,280 g (controls), 22,10±1,26 g, 18,20±1,26 g and 12,63±1,29 g (1-rst, 2-nd and 3-rd group, respectively) on the 6-th gestational day. On the 12-th gestational day, the weight gain in pregnant dams were 59,80±2,31 g (controls), 42,7±2,96 g, 29,30±2,18 g and 18,93±1,37 g (1rst, 2nd and 3rd group, respectively). On the 18-th gestational day, the weight gain in pregnant rats were 79,70±2,68 g (controls), 57,5±2,68 g, 45,10±2,26 g and 39,30±2,09 g (1rst, 2nd and 3rd group, respectively). The results of weighting of rats were 88,30±3,52 g (controls), 61,11±1,67 g,

58,70±2,93 g and 47,80±2,03 g (1rst, 2nd and 3rd group, respectively) on the 20-th gestational day. A treatment effect of Laprol-604 was significantly different indicated in 3-rd and 2-nd groups compared with controls, with the exception of the 1-rst group (0,125 mg/kg) group. Significant variations from controls for the 12,5 mg/kg dose group beginning at 6 gestational day (GD), the 1,25 mg/kg dose group at from GDs 12 to 18. The significant reduction of food consumption was noted but water intake was increased in these groups of pregnant rats. Laprol-604 showed dosage dependent developmental toxicity when the pregnant rats were exposed. It led to reduced statistically significant body weight of pregnant dams. The external developmental anomalies of the facial and cerebral skull, eyes, ear shells, anterior abdominal wall, limbs, tail were not detected while fetus bodies were being examined.

The exposure to Laprol-604 throughout gestation period produced adverse effects on the number of live fetuses: 89,10±4,80% (controls), 84,80±2,60%, 76,80±3,50%, 54,80±4,90% (1rst, 2nd and 3rd group, respectively). The evaluation of fetus materials showed, on the one hand, the number of yellow bodies of pregnancy, the fetal weights were reduced by Laprol-604, on the other hand, the amount of resorptions, preimplantic, postimplantic and total fetal death were increased. For example, the fetal weights were 3,97±0,14 g (controls), 3,56 ± 0,15 g, 2,90 ± 0,16* g and 2,20 ± 0,14* g (1rst, 2nd and 3rd group, respectively). The fetal death before implantation 5,30 ± 0,26 (controls), 7,40 ± 0,8, 9,30 ± 0,60* and 13,20 ± 1,05* (1rst, 2nd and 3rd group, respectively). The fetal death after implantation 3,30 ± 0,35 (controls), 5,20 ± 0,65, 7,60 ± 0,73* and 9,30 ± 0,6* (1rst, 2nd and 3rd group, respectively). In addition, weights of placentas were increased such 0,41 ± 0,13 g (controls), 0,63 ± 0,05 g, 0,67 ± 0,08* g and 0,78 ± 0,06* g (1rst, 2nd and 3rd group, respectively).

The teratogenic effects such as skeletal abnormalities: cleft palate (%) was not detected, sternal defects (per fetus) were found in 1,2 ± 0,3 (controls), 1,7 ± 0,3, 2,1 ± 0,3 and 4,9 ± 0,4* (1rst, 2nd and 3rd group, respectively), between the 3-rd and control groups the data were significantly differenced. Notable visceral defects such as anasarca, enlarged right atrium and ventricular septal defects were not revealed, during examination of rat fetuses. On the other hand delayed ossification of fetus phalanges were noted, concerning ossified proximal phalanges (per fore limb) were 3,46 ± 0,3 (controls), 2,89 ± 0,4, 2,60 ± 0,4 and 1,8 ± 0,2* (1rst, 2nd and 3rd group,

respectively); concerning ossified proximal phalanges (per hindlimb) 4,90 ± 0,1 (controls) 4,10 ± 0,6, 3,80 ± 0,6 and 2,1 ± 0,3* (1rst, 2nd and 3rd group, respectively). The delayed ossification of proximal phalanges of fetuses the 3-rd group were significantly different from controls.

Discussion. Maternal toxicity of Laprol-604, indicated by deficits in weight gain was observed in 1-rst, 2-nd and 3-rd grpups, during pregnancy. In all groups, the severity of the Laprol-604 adverse effects were dose-dependent. The lag in weight gain was particularly pronounced in the 3-rd group (the highest dosage group, 12,5 mg/kg), which exhibited reduction of food intake but water consumption was increased, during pregnancy. The Laprol-604-induced reductions of weight gain in the pregnant rat seen here are comparable to similar alterations produced by the non-ionic surfactant in the rats and mice [22], indicating that the adverse effect on maternal weight gain may be a common feature of toxicity for the non-ionic surfactants. Liver enlargement with associated histological abnormalities is another feature seen after exposure to Laprol-604 and other non-ionic surfactants [18, 19, 22]. A somewhat similar finding was obtained in another study [22]. An increase of liver weight is generally observed in rodents during pregnancy (by about 24% in rat). Above and beyond this physiological change, significant elevations of hepatic weight were found in the 3-rd group as much as twofold over the corresponding the rat of control group. Interestingly, the small increase in the relative liver weight in the 0,125 mg/kg dosage group largely reflected the reduction of body weight, rather than a net increase of liver weight. Nonetheless, the high sensitivity to Laprol-604-induced liver toxicity in the rat of 3-rd group should be noted and the liver weight increase estimated at 2,61 mg/kg. On the other hand, anasarca, craniofacial malformation (cleft palate), cardiac defects (ventricular septal defects, enlargement of the right atrium), and delayed ossification (phalanges) were not detected in the Laprol-604-exposed fetuses. These results are in agreement with previous teratological findings with another non-ionic surfactant Laproxide-303 in the rat. Although a significant reduction of weight gain and food consumption was noted, but water intake was increased in these groups of pregnant rats.

The liver is such a frequent target organ in toxicity studies (in fact, the most common) that a discussion of some of the more common lesions that occured in the pregnant rats seems warranted. Observations carried out on the pregnant rats, their fetuses, lead to the following findings: continuous increase of liver weight along

pregnancy from 2nd to 20th days, mainly within the 2nd week (organs making), to reach limit value on the 18th day. This liver weight increases roughly along with amount of embryos and placentas weights specially from 14th to 20th days. At the 18th day the mother's liver weight depends on amount embryos and placentas weights. This weight of the mother's liver increased with embryos and placentas number but does not depend on resorption number. The overall mother's weight is proportional to liver weight and embryos number in pregnant rats of control group. All these facts underline metabolic relations between mother and fetus during pregnancy.

In view of the profound deficits in maternal weight gain in Laprol-604-exposed rats, it was surprising to find little adverse effect on the viability of the fetuses. In fact, only small decrements of fetal weight were noted. Similar results were obtained with the mouse, even at higher exposures. On the other hand, delayed ossification (phalanges) were detected in the Laprol-604-exposed fetuses. These results are in agreement with previous teratological findings with polyols such as P-373-2-20, P-5003-AC and P-294-2-35 in the Wistar rats, mice and Guinea pigs. Although Laprol-604 did not induce of of birth defects, nonetheless, evaluation of embryonic and fetuse materials obtained at autopsy pregnant Wistar rats showed the fetal weights were reduced by Laprol-604 and the amount of resorptions, preimplantic, postimplantic places and total fetuses death were increased. It should be noted that a preponderance of these abnormalities was found in the highest Laprol-604 dosage 3-rd group (12,5 mg/kg). A significant reduction of weight gain and food consumption was noted in 3-rd group of pregnant rats. Indeed, equivalent or higher incidence of reducing weights was seen in the fetuses of 3-rd group as well as the deficits of weight gain and food consumption in the rat dams of 3-rd group were much less extensive than those of the pregnant rats of 2-nd and 1-rst groups.

This study coupled with increased exposure to daily use of surfactants. The importance of identifying and characterizing the reproductive risks of Laprol-604 intended for using by reproductive age population. These risks broadly divided into two categories, reproductive risks and developmental risks. The reproductive risks are related to impact on processes like fertility (male and female), giving birth and lactation. The developmental risks are related to the fetus and include mortality, alteration in growth and functional deficits. On the one hand, the teratogenic effects were not detected in fetuses, on the other hand, the significant reduction of weight

gain and food consumption in the all Laprol-604 administration group. These findings are consistent with the results of other studies of non-ionic surfactants employing a different dose-regimen of surfactant. The pathophysiological mechanisms underlying Laprol-604-induced are largely unknown at present. However, among the available data [18] reported a similar pattern of neonatal death that was explained by numerous structural metabolic disorders, as well as possibility to condition remote consequences.

Conclusion. In view of the profound deficits in maternal weight gain in 12,5 mg/kg of Laprol-604-exposed rats, it was expectable to find adverse effect on the viability of the fetuses. In fact, the decrements of fetal weight were noted. Similar results were obtained with the fetuses of 2-nd group. Although Laprol-604 did not show teratological effects, but, jn the other hand, delayed ossification (sternebrae, phalanges) were detected in the Laprol-604-exposed fetuses.

Acknowledgement. If Laprol-604 is intended for long-term use, it will be needed to study the offspring be examined for possible adverse effects on later development, behavior and reproductive capacity.

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