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The Dow Chemical Company
Midland, Michigan 48674
USA

April 27, 2009

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[CONTAINS NO CONFIDENTIAL BUSINESS INFORMATION]

Document Processing Center (7407M)
(Attn: TSCA Section 8(e) Coordinator)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

Re: Ethanol, 2,2'-iminobis- (diethanolamine) CASRN 111-42-2

Dear Sir/Madam:

The following information is being submitted by The Dow Chemical Company (Dow) pursuant to current guidance issued by EPA indicating EPA's interpretation of Section 8(e) of the Toxic Substances Control Act. Dow has made no determination as to whether a significant risk of injury to health or the environment is actually presented by the findings.

Dow became aware of an abstract from the Teratology Society Poster Session that summarized reproductive and developmental toxicity studies of diethanolamine. Subsequently Dow received a copy of the full paper (in Korean), *A Study on the Reproductive and Developmental Toxicity of Diethanolamine*, from The Annual Report of KFDA, Vol. 11, 1802 – 1824, 2007. A copy of the abstract, the original paper in Korean and an unofficial English translation of the paper is attached.

Questions may be addressed to the undersigned.

Sincerely,

Imogene E. Treble

8EHQ-09-17501

Imogene E. Treble
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Contains No CBI

jt

Attachments

CONTAINS NO CB

8809000225

MR 318886

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LEE RD, RHEE GS, MOON HJ, SEOK JH, YANG JY, CHAE SY, LEE YU, CHUNG SY. National Institute of Toxicological Research, Seoul, Republic of Korea. Study on the Reproductive and Developmental Toxicity of Diethanolamine

Diethanolamine (DEA) is widely used as a chemical intermediate, as an anticorrosion agent in metalworking liquids, and as a surface-active agent in cosmetic formulations, pharmaceuticals, and agricultural products. Estimated annual production of DEA in the U.S. was 106,000 tons in 1995. The most probable route of environmental exposure to DEA in humans in via dermal exposure to personal care products, detergents, and other surfactants that contain DEA; cosmetic formulation mayhave concentrations of DEA ranging form 1 to 25%. The National Institute for Occupational Safety and health estimates that the number of worker potentially exposed to DEA is ~800,000/year. However little is known about their potential toxicological properties. The purpose of our study is to investigate the paternally and maternally mediated effects including fertility, physical development, reflex and behaviors after exposure to DEA (0, 20, 80, 320 mg/kg), respectively. Also DEA-induced developmental abnormalities were investigated *In vitro*. Postimplantation embryos at GD 9.5 were cultured for 48 h and observed for morphological changes. DEA mediated changes in proliferation and differentiation were measured in embryonic midbrain and limbbud cells. In paternally mediated effects study, Sperm motility and testes organ weight of F1 mice born of F0 male treated with diethanolamine was reduced significantly. In maternally mediated effects study, DEA showed external abnormalities of fetuses of offsprings and the body weight gain, physical development of F1 mice were influenced by DEA. *In vivo* study, reflex, movement and learning/memory functions were changed by treatment with DEA, approximately. Altogether, it is suggested that treatment of DEA in this study for reproductive and developmental toxicities have adverse effects on offsprings and alter learning ability.

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BENASSI JC, GÓRNIAK SL, HUEZA IM. School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, SP, Brazil. Evaluation of the Possible Immunotoxic Effects of Monocrotaline on the Offspring of Female Rats Treated During the Gestation

Introduction: Monocrotaline (MCT) is a pyrrolizidinic alkaloid founded in many poisonous plants widely distributed in the world. Natural intoxication with these plants occurs when livestock invade areas where they are cultivated as "green fertilizer". Moreover, human foodstuffs, such as milk, honey, grains, herbal medicines and dietary supplements, can be also contaminated by MCT. To be toxic, MCT must be metabolized in the liver into a pyrrole compound (MCTP). The main toxic effects of MCT are hepato- and nefrotoxicity, in rodents pulmonary hypertension is also observed. The mechanism of toxicity of MCTP is related to its DNA affinity, resulting in DNA adduction, leading to an antimitotic state and cancer development. Due to this antiproliferative effect, it is possible to suggest that MCT exposure in uterus can compromise both fetus viability and the immune system development. Evaluate the possible teratologic effect of MCT. Methods: Twenty one pregnant rats were distributed into 1 control and 2 experimental groups, which received 0, 3 and 5mg/kg of MCT, orally, from GD6 to 21. Clinical signs, body weight gain (BWG) and food intake (FI) were measured daily. Immediately after birth, pups were evaluated for gross alterations and weighed until postnatal day (PND)7. On PND 4 and 7, half of each litter was killed to lymphoid organs evaluation (thymus and spleen). Results: No clinical signs of MCT toxicosis were observed in both dams and pups. Rats treated with MCT did not show reduction in either BWG or FI. No difference in BW was noticed in any group of pups immediately after birth; however, female pups from dams treated with 5mg/kg showed a reduced BWG on PND4. Interestingly, only on PND7 it was observed a decreased in thymus relative weight of both gender of pups from female treated with 3mg/kg of MCT. Male pups from 3mg/kg treatment showed splenomegaly on PND7. Conclusion/Discussion: For our knowledge, the present work is the first to describe that MCT exposure in uterus can compromise immune organs, especially thymus, one of the most sensitive lymphoid organs to xenobiotic exposure. Despite of the discrepancies among data from gender and period of life of pups, the dose here employed can be consider as not toxic, as could be seen in mother parameters. Thus, experiments have been conducted in our lab, employing higher doses of MCT, to better verify the effect of this alkaloid on the developing immune system. FAPESP 07/51648-0.

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식품의약품안전청연구보고서 제 11 권 The Annual Report of KFDA, Vol.11, 1802~1824. 2007

디에탄올아민 (Diethanolamine)의 생식발생독성에 관한 연구

국립독성과학원 생식독성과

이이다, 이규식, 문현주, 석지현, 양준영, 채수영, 이여울, 김수미, 김승희 정수연*

국 문 요 약

디에탄올아민 (diethanolamine, DEA)은 샴푸, 린스, 비누, 자외선 차단제, 화장품, 헤어스프레이 및 파마 (염색)약 등 일반생활용품과 의약품 및 농약 등에 유화제, 침윤제, 계면활성제 및 농축제 등으로 널리 사용되고 있다. 디에탄올아민은 피부로 쉽게 흡수되어 위암, 식도암, 간암, 방광암 등을 유발할 수 있는 발암물질이라는 지적이 제기되어 주목되었고, 최근 임산부가 디에탄올아민에 노출시 유산 및 태아의 뇌 형성 과정에 유해한 영향을 미쳐 기억 및 학습장애에 영향을 미친다는 보고와 함께 남성의 정자발생에도 관여하여 기형출산 등의 가능성 또한 제시되고 있으나, 생식발생독성 및 후세대에 미치는 영향에 대한 구체적인 실험결과가 보고된 바 없다. 본 연구에서는 일반생활용품에 다량 함유되어 있어 그만큼 노출가능성이 높은 디에탄올아민의 부체, 모체 각각 노출에 의한 유해영향을 규명하고자 하였다. 부체 노출에 의한 영향을 알아보고자, 디에탄올아민 (0, 20, 80, 320 mg/kg)을 4주간 수컷에게 도포한 후 정상 암컷과 교미시켜 차산자를 얻었으며, 모체 노출에 의한 영향을 알아보고자, 임신 중 디에탄올아민을 도포하여 차산자를 출산한 후 장기중량 및 수태능, 신체발육 · 반사기능, 운동 · 학습 · 기억 능력 등을 평가하였다. 연구결과, 디에탄올아민의 부체 노출에 의해 태어난 차산자의 부고환 중량이 유의적으로 감소하였으며, 통각시험에서도 용매대조군에 비하여 매우 유의적으로 느린 반응을 나타내었다. 디에탄올아민의 부체 노출에 의해 태어난 차산자의 경우는 암컷 차산자의 체중 증가율이 유의적으로 감소하였으며 운동능력 또한 용매대조군에 비하여 현저히 떨어지는 것을 확인하였다. 부체 및 모체 각각 노출시험 모두에서 운동 · 학습 · 기억 능력 등은 대체적으로 디에탄올아민에 의해 감소하는 경향을 보였으며, 특히 정자운동성의 경우 모체 노출군에서는 약간의 감소 경향만을 보였으나 부체와 부체 노출에 의해 태어난 차산자는 현저하게 유의적으로 감소함을 나타내었다. 향후 디에탄올아민의 후세대영향 연구를 위하여 2세대 이후로 확대 수행하여야 하며, 디에탄올아민이 나타내는 생식발생독성 기전을 규명하여야 할 것으로 판단된다.

검색어 : 디에탄올아민, 생식발생독성, 후세대영향

서 론

디에탄올아민 (diethanolamine, DEA)은 샴푸, 린스, 비누, 자외선 차단제, 화장품, 헤어스프레이 및 파마 (염색)약 등 일반생활용품과 의약품 및 농약 등에 유화제, 침윤제, 계면활성제 및 농축제 등으로 널리 사용되고[1,2] 미국에서만 매년 10만톤 이상 사용되고 있다[3]. 디에탄올아민은 피부로 쉽게 흡수되어 위암, 식도암, 간암, 방광암 등을 유발할 수 있는 발암물질이라는 지적이 제기되어 주목 되어왔다. 특히 샴푸의 경우 풍부한 거품을 만들어 내기 위한 목적으로 diethanolamine이 제품에 함유되는 전체 화학물질의 25%에[3] 달하는 경우도 있다는 전문가들의 지적이 있음에도 생식발생독성에 대한 유해정보는 극히 제한적이다. 최근 임산부가 diethanolamine에 장기노출시 유

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산 및 태아의 뇌 형성 과정에 유해한 영향을 미쳐 기억 및 학습장애에 영향을 미친다는 보고[4]와 함께 남성의 정자발생(spermatogenesis)에도 관여하여 기형출산 등의 가능성 또한 제시[5] 되고 있다. 하지만 정확한 독성기전은 물론 생식발생독성 및 후세대에 미치는 영향에 대한 구체적인 실험결과는 아직까지 보고된 바가 없다. 따라서 일반생활용품에 다량 함유되어 있어 그만큼 노출 가능성이 높은 diethanolamine의 부모세대에 미치는 유해영향에 대한 전면적인 평가와 더불어 생식발생독성시험 표준시험법을 적절히 조합한 *in vivo* 시험을 수행하고, 연구결과에 기초하여 diethanolamine의 위해성을 규명하고 향후 후세대 건강을 보호하는데 기여하고자 한다.

재료 및 방법

디에탄올아민에 의한 생식발생독성을 알아보고자 부체 (수컷) 노출에 의한 차산자의 독성영향 및 모체 (암컷) 노출에 의한 차산자의 독성영향으로 나누어 수행하였다.

1. 생식발생독성시험

가. 부체노출

5주령의 수컷 C57BL/6 마우스를 동물용 제모기를 이용하여 견갑골 부위의 털을 제거한 후 디에탄올아민 0, 20, 80, 320 mg/kg 용량으로 군당 15수씩 매일 오전 동일한 시각에 4주간 피부에 도포하였다. 4주간 디에탄올아민의 도포가 끝난 처리군과 대조군은 9주령의 암컷 C57BL/6 마우스 각각 1:1로 동거시켰으며 다음날 질전 검사를 하여 질전 (vaginal plug)이 확인되면 임신 0일로 정하였다.

나. 모체노출

9주령의 암·수컷 C57BL/6 마우스 각각 1:1로 동거시켰으며 다음날 질전 검사를 하여 질전 (vaginal plug)이 확인되면 임신 0일 (GD 0)로 정함으로써 임신 모체를 준비하였다. 임신 6일부터 동물용 제모기를 이용하여 견갑골 부위의 털을 제거한 후 디에탄올아민 0, 20, 80, 320 mg/kg 용량으로 군당 10수씩 매일 오전 동일한 시각에 1회씩 도포하고 분만일을 분만 0일 (postnatal day, PND 0일)로 하고 PND 21일까지 계속 모체에 도포하였다. PND 4일에 차산자를 암수 각각 4마리씩 조정하고, 이유 후, 모체와 차산자 (암·수 각각 2수씩) 부검하고 나머지는 행동발달 사항을 검사하기 위해 계속 사육하였다.

다. 공통사항

특정병원체부재 (SPF) C57BL/6 마우스 암·수컷을 (주)오리엔트 (Seoul, Korea)로부터 구입하고 미국 실험동물관리 인증협회 (Association for Assessment and Accreditation of Laboratory Animal Care; AAALAC)가 인증한 식품의약품안전청 국립독성과학원 청정사육시설을 이용하였다. Diethanolamine은 Sigma 사 (St. Louis, MO, U.S.A. D0681, 98.5%), ethanol은 Merck 사 (Germany)의 제품을 사용하였고, ethanol을 용매로 하여 diethanolamine 20, 80, 320 mg/kg 용량으로 매일 오전 동일한 시각에 견갑골 사이의 2 ㎠ 정도에 도포하였으며, 대조군 역시 동일한 부위에 ethanol을 도포하였다. 견갑골 사이의 도포부위는 동물용 제모기를 이용하여 털을 제거하였다 (figure 1).

각각 차산자 (F1) 암·수컷의 체중은 출생일 (PND, postnatal day) 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70일에 측정하였고, 부체, 모체 및 차산자의 사료섭취량은 주 1회씩 측정하여 증가분을 조사하였다. 수컷과 암컷 마우스를 각각 1:1로 동거시켰으며 다음날 질전 검사를 하여 질전 (vaginal plug)이 확인되면 임신 0일로 정하였다. 임신 유무의 최종 판정은 부검 시 자궁 내 태자의 존재, 착상흔적 또는 자연 분만 시 태자의 출산에 의해 판단하였다. 노출 부체 및 임신 중 노출 모체를 부검하고 임신 18일 태자의 골격이상 등을 평가하였다. 또한 나머지 차산자의 출생일 21일에 이유하여 부검하였다.

Figure 1. Dermal application

라. 차산자 평가

전 시험군에 대하여 시험기간 중 1일 1회 동물의 일반증상 및 사망유무를 관찰하고, 이상이 있으면 증상의 종류, 발견일과 시각, 지속기간 및 필요에 따라서는 증상의 정도를 기록하였다.

차산자의 발생 및 발달상태를 보기 위해 출생일 4일째 (PND 4)에 모체 한 마리당 차산자 암·수 각각 4수씩 총 8수로 조정하였으며, 출생당시 차산자 수와 조정 후 차산자 수를 비교하여 기록하였다. 출생일 21일 (PND 21)에 이유시키고, 모체 당 암수 차산자 각각 2수 이상 씩 부검하여 일반장기 및 생식장기를 비교하였으며, 육안적 병변부위는 10% 중성포르말린 용액에 고정하여 보존하였다.

이유기부검이 끝나고 남은 나머지 차산자는 성장, 발육 및 행동발달을 확인하기 위해 출생 4일째에 감각모 (Sensory hair)와 이개개전 (Pinna detachment), 7일째에 피모발생 (Visible pilation)와 이개반사, 9일째에 정향반사 (Surface righting reflex)와 부주지성 (Negative geotaxis), 10일째에 치아맹출 (Incisor eruption)과 발톱성장 (뒷발, Growth of claw), 14일째에 위치정립반사 (Midair righting reflex), 절애회피 (Cliff avoidance), 견인력시험 (Wire grasping) 및 안검개열 (Eye opening), 20일째에 각막반사와 정소하강 (Testis descent), 28일째에 회전봉시험 (Rotarod test), 30일째에 질개구 (Vaginal opening), 35일째에 자발운동량 (Open field), 42일째에 수동회피시험 (Passive avoidance test), 45일째에 통각시험(Hot plate test) 및 63일째에 공포량 측정시험 (Fear conditioning test) 등을 관찰 및 측정하였다

모든 성장, 발육 및 행동발달 검사가 끝난 성숙기 (PND 70) 암·수컷 차산자의 일반장기 및 생식장기의 중량을 측정하고, 장기와 육안적 병변부는 10% 중성포르말린용액에 고정하여 보존하였다.

마. 정자운동성 측정

DEA에 노출된 부체와 부체 및 모체 노출에 의해 생산된 각각의 수컷 차산자에 대한 정자운동성 및 속도 등을 Hamilton-Thorne IVOS 2000 system을 이용하여 측정하였다.

2. 통계학적 해석

ANOVA test 후 Dunnett's test를 이용하여 $p<0.05$의 수준에서 용매대조군과 시험물질 투여군 간의 유의성을 검정하였다.

결과 및 고찰

가. 부체노출에 의해 차산자에 미치는 독성평가

디에탄올아민 (0, 20, 80, 320 mg/kg)을 4주간 견갑골 부위에 도포하는 동안 모든 투여군에서 시험물질 투여로 인한 임상증상을 관찰되지 않았으며, 사망동물 또한 발생하지 않았다. 고용량에서 도포부위에 각화현상이 관찰되었으나 바로 회복되었다.

부체의 경우 디에탄올아민을 4주간 투여하는 동안 마우스의 체중변화에 있어서 용매대조군과 시험물질 투여군간에 차이는 발생하지 않았고, 유사한 증가율을 보였다. 부체가 디에탄올아민에 노출되어 출생한 암·수 차산자의 체중은 암컷 차산자의 경우 용매대조군과 비교시 차이가 관찰되지 않았으며, 수컷 차산자의 체중변화는 고용량 (320 mg/kg) 군의 경우 용매대조군과 비교하여 다소 감소하는 경향을 나타내었으나 유의성은 관찰되지 않았다 (Table 1, Figure 2).

4주간의 디에탄올아민 투여 후 부검결과 용매대조군 및 시험물질 투여군의 모든 동물에서 신체 전 장기의 이상소견이 관찰되지 않았다.

뇌, 간, 비장, 신장 등 주요 일반장기와 고환, 부고환 등 생식장기를 적출하여 중량을 측정한 결과 부고환 중량의 경우 용매대조군과 비교 시 고용량으로 갈수록 약간 감소하는 경향을 보이나 유의성을 없었다 (Table 2). 암·수컷 차산자를 이유기 (PND 21)와 성숙기 (PND 70)에 부검하여 일반장기와 생식장기를 적출하여 중량을 측정한 결과, 수컷 차산자 이유기 부검시 디에탄올아민 80 mg/kg 용량에서 부고환의 중량이 유의성 있게 감소하였으며, 암컷 차산자는 자궁 중량이 증가하는 경향을 나타내었다 (Table 3, 4). 성숙기 부검을 실시한 결과, 최고용량 320 mg/kg 군에서 수컷 생식장기의 중량이 유의적으로 감소하

였으며 암컷의 경우도 이유기 부검과 비슷한 양상으로 자궁의 중량이 증가하는 경향을 보였다 (Table 5, 6).

임신 18일에 제왕절개하여 적출한 생존태자의 내장 및 골격이상에 대한 검사결과 용매대조군과 디에탄올아민 투여군 간의 큰 차이는 관찰되지 않았으며, 암·수컷 차산자 성장, 발육 및 행동발달을 확인하기 위해 시기별로 신체발육평가 (감각모, 이개개전, 안검개열, 치아맹출, 발톱성장, 정소하강, 질개구 등), 반사기능 (이개반사, 각막반사), 자세제어기능 (정향반사, 부주지성) 등을 관찰한 결과 고용량군에서 용매대조군에 비해 유의성이 확인되지는 않았으나 피모발생이 늦어졌으며 또한 부주지성이 감소함을 알 수 있었다. 또한 수컷 성성숙을 나타내는 지표인 정소하강이 용매대조군에 비하여 고용량군에서 늦어지는 것으로 나타났다 (Table 7). 통각시험 (Hot-plate test)에서는 용매대조군에 비하여 현저하게 용량의존적으로 유의성 있는 증가를 나타내었다 (Table 8 & Figure 3).

암·수컷 차산자의 운동능력 (rota-rod test)을 측정한 결과 수컷의 경우 20 mg/kg 군에서만 약간 감소하였고, 암컷은 고용량에서 감소하는 경향을 나타내었으나 유의성은 없었다 (Table 9 & Figure 4). 시간당 정해진 공간에서의 운동량을 측정하는 자발운동량 (open field)을 측정한 결과 용매대조군에 비하여 암·수컷 모두 별다른 차이가 관찰되지 않았다 (Table 10 & Figure 4). 소음과 전기충격을 주어 인지능력을 평가하는 시험인 공포량측정시험 (fear conditioning test) 결과 암·수컷 모두 용매대조군에 비하여 감소하는 경향을 나타내었으나 유의성은 없었다 (Table 11 & Figure 4). 전기충격을 주어 그 상황을 인지하는 능력을 검사하는 수동회피시험 (passive avoidance test) 결과도 마찬가지로 암·수컷 모두 용매대조군에 비하여 감소하는 경향을 나타내었으나 유의성은 없었다 (Table 12 & Figure 4).

디에탄올아민이 부체를 통해 수컷 차산자의 생식능력에 미치는 영향을 알아보고자 부고환의 정자운동성 및 정자속도 등을 용매대조군과 비교함으로써 관찰하였다. 부체의 경우 용매대조군에 비해 정자운동성이 현저하게 용량의존적으로 감소하여 유의성을 나타내었으며, 차산자 역시 용량의존적인 감소경향을 보였으며 특히 고용량 320 mg/kg 에서는 유의성이 있었다 (Table 13, 14 & Figure 5).

나. 모체노출에 의해 차산자에 미치는 독성평가

디에탄올아민 (0, 20, 80, 320 mg/kg)을 임신 중 및 수유기에 견갑골 부위에 도포하는 동안 모든 투여군에서 시험물질 투여로 인한 임상증상이 관찰되지 않았으며, 사망동물 또한 발생하지 않았다. 고용량에서 도포부위에 각화현상이 관찰되었으나 바로 회복되었다.

임신 중 디에탄올아민에 노출되는 동안 임신 모체의 체중변화에 있어서 용매대조군과 시험물질 투여군간에 차이는 발생하지 않았고, 유사한 증가율을 보였다. 임신 중 디에탄올아민에 노출된 모체에서 태어난 암·수 차산자의 체중은 용매대조군에 비해 유의적으로 감소함을 나타내었으며 특히 암컷 차산자의 경우 최고 용량에서 출생 후 7일부터 49일까지 특히 용매대조군에 비하여 체중이 유의적으로 감소하였다 (Table 15 & Figure 6).

임신 중 및 수유기에 디에탄올아민 투여 후 부검결과 용매대조군 및 시험물질 투여군의 모든 동물에서 신체 전 장기의 이상소견이 관찰되지 않았다.

뇌, 간, 비장, 신장 등 주요 일반장기와 고환, 부고환 등 생식장기를 적출하여 중량을 측정한 결과 대조군과 비교시 80 mg/kg 용량군에서 자궁의 중량이 증가하였으나 유의적이지는 않으며, 일반장기는 대체로 약간의 증가경향을 나타내었다 (Table 16). 암·수컷 차산자를 이유기 (PND 21)와 성숙기 (PND 70)에 부검하여 일반장기와 생식장기를 적출하여 중량을 측정한 결과, 암·수컷 차산자 모두 이유기 부검시 용매대조군과 비교하여 차이점이 나타나지 않았으며, 암컷 차산자 성숙기에 부검한 장기중량의 결과도 특이한 점은 없었다. 단지 수컷 차산자 성숙기 부검시 투여군에서 부신의 중량이 유의적으로 감소함을 나타내었다 (Table 17-20).

임신 18일에 제왕절개하여 적출한 생존태자의 내장 및 골격이상에 대한 검사결과 용매대조군과 디에탄올아민 투여군 간의 큰 차이는 관찰되지 않았으나 정확한 판독을 위한 관찰실험이 현재 진행 중에 있다.

암·수컷 차산자 성장, 발육 및 행동발달을 확

인하기 위해 시기별로 신체발육평가 (감각모, 이개개전, 안검개열, 치아맹출, 발톱성장, 정소하강, 질개구 등), 반사기능 (이개반사, 각막반사), 자세제어기능 (정향반사, 부주지성) 등을 관찰한 결과 유의성이 확인되지는 않았으나 수컷 성성숙을 나타내는 지표인 정소하강이 용매대조군에 비하여 디에탄올아민 노출군에서 늦어지는 것으로 나타났다 (Table 21). 통각시험 (Hot-plate test)에서는 용매대조군에 비하여 용량의존적으로 증가하는 경향을 나타내었다 (Table 22 & Figure 7).

암·수컷 차산자의 운동능력 (rota-rod test)을 측정한 결과 암·수컷 모두 용량의존적으로 감소하였으며 특히 수컷 처리군 모두 용매대조군에 비하여 유의적으로 감소함을 나타내었다 (Table 23 & Figure 8). 시간당 정해진 공간에서의 운동량을 측정하는 자발운동량 (open field)을 측정한 결과 용매대조군에 비하여 암·수컷 모두 별다른 차이가 관찰되지 않았다 (Table 24 & Figure 8). 소음과 전기충격을 주어 인지능력을 평가하는 시험인 공포량측정시험 (fear conditioning test) 결과 암·수컷 모두 용매대조군에 비하여 감소하는 경향을 나타내었으나 유의성은 없었다 (Table 25 & Figure 8). 전기충격을 주어 그 상황을 인지하는 능력을 검사하는 수동회피시험 (passive avoidance test) 결과도 마찬가지로 암·수컷 모두 용매대조군에 비하여 감소하는 경향을 나타내었으나 유의성은 없었다 (Table 26 & Figure 8).

임신 중 및 수유기에 디에탄올아민에 노출된 모체를 통해 수컷 차산자의 생식능력에 미치는 영향을 알아보고자 부고환의 정자운동성 및 정자속도 등을 용매대조군과 비교함으로써 관찰하였다. 수컷 차산자는 유의성이 나타나지는 않았으나 정자운동성에서 감소경향을 보였다 (Table 27 & Figure 9).

임신 중 디에탄올아민에 노출된 모체에서 태어난 수컷 차산자는 정소하강 및 정자운동성이 다소 늦어지는 경향을 보였으나, 부체 뿐만 아니라, 부체가 디에탄올아민에 노출되어 정상 암컷과의 교미에 의해 출생한 수컷 차산자는 부고환 중량이 유의적으로 감소하였으며 정자운동성 역시 고용량군에서 유의적으로 감소하는 것으로 나타났다. 이는 모체 노출 보다는 부체 노출이 차세대의 정자발생에 더욱 큰 영향을 줄 것으로 판단되며 정자이상은 선천성 기형을 유발할 수 있으므로 관련 연구가 수행되어야 할 것으로 본다.

또한 임신 중 디에탄올아민에 노출된 차산자는 신체발육, 반사기능, 운동·학습·기억 능력 등이 현저히 떨어지는 것을 확인할 수 있었을 뿐 아니라, 부체 노출에 의해서도 동일한 양상이 나타났다. 이러한 결과는 모체 노출 없이도 부체 노출만으로 후세대에 영향을 줄 수 있다는 사실을 시사하고 이에 대한 관련 연구가 확대 수행되어야 할 것으로 판단된다.

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Table 1. Body weight of F_1 generation mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)		Control	20	80	320
PND4	Male	3.20±0.34	3.34±0.26	3.01±0.36	3.11±0.57
	Female	3.23±0.45	3.27±0.15	2.65±0.65	2.94±0.42
PND7	Male	4.67±0.53	5.19±0.27	4.36±0.59	4.71±0.35
	Female	4.72±0.67	5.00±0.13	4.18±0.68	4.78±0.27
PND14	Male	7.51±0.95	8.49±0.34	7.41±0.78	7.48±0.48
	Female	7.46±0.86	8.18±0.48	7.00±0.93	7.49±0.38
PND21	Male	11.06±1.13	11.21±0.47	10.16±1.01	10.00±1.88
	Female	10.81±1.11	10.82±0.58	9.51±1.34	10.70±0.75
PND28	Male	15.54±1.94	15.90±0.95	15.18±1.12	14.56±2.50
	Female	14.24±1.60	14.72±0.87	12.95±1.45	14.18±1.01
PND35	Male	18.64±1.47	19.20±1.33	18.85±0.61	17.90±2.47
	Female	16.62±1.24	16.69±1.58	15.80±1.34	16.51±1.28
PND42	Male	20.24±1.23	20.98±1.41	20.43±0.93	19.40±2.27
	Female	18.29±1.34	18.36±1.81	17.01±1.17	17.67±1.31
PND49	Male	21.08±1.56	21.49±1.60	21.42±1.01	19.23±3.78
	Female	18.76±1.49	18.76±1.61	17.55±1.20	18.20±1.49
PND56	Male	22.18±1.90	22.68±1.54	22.59±1.05	21.43±2.12
	Female	19.76±1.70	19.48±1.60	18.39±1.31	19.04±1.71
PND63	Male	23.27±1.55	23.50±1.48	23.52±1.17	22.03±2.36
	Female	20.41±1.77	20.22±1.81	18.95±1.23	19.72±2.02
PND70	Male	23.93±1.55	24.85±2.38	24.50±1.36	22.84±2.31
	Female	21.20±1.70	20.87±1.81	19.74±1.41	20.45±1.90

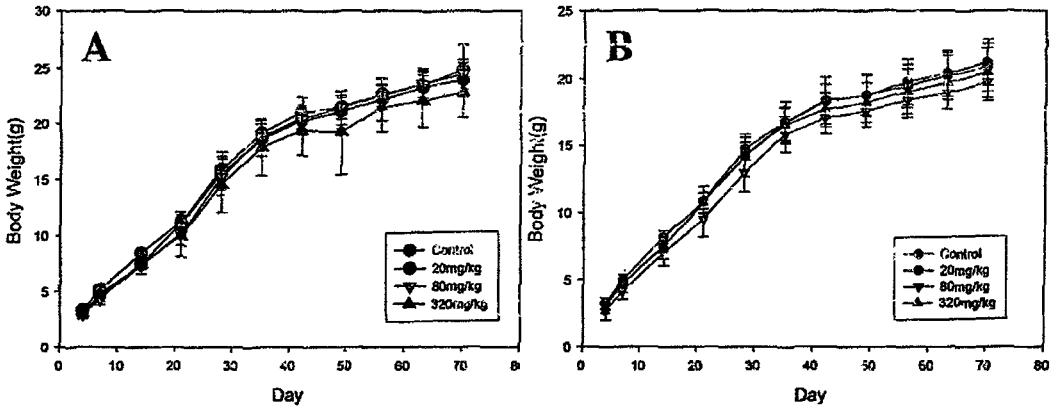

Figure 2. Change in body weight of F_1 male (A) and female (B) mice born of F_0 male treated with diethanolamine

Table 2. Absolute organ weights (g) of F_0 male mice treated with diethanolamine

Treatment (mg/kg)	Control	20	80	320
Final Body Weight	34.10±3.34	35.17±2.51	33.88±4.19	33.36±3.25
Brain	0.4316±0.0120	0.4395±0.0189	0.4136±0.0339	0.4318±0.0319
Liver	1.4469±0.1281	1.4919±0.1354*	1.3765±0.1350	1.3897±0.1405
Kidney	0.3741±0.0202	0.4019±0.0227	0.3960±0.0337	0.3737±0.1136
Thymus	0.0346±0.0047	0.0363±0.0070	0.0365±0.0045	0.0372±0.0037
Spleen	0.0567±0.0087	0.0587±0.0066	0.0561±0.0206	0.0611±0.0079
Adrenal gland	0.0031±0.0006	0.0031±0.0006	0.0033±0.0009	0.0035±0.0004
Testis	0.1546±0.0072	0.1605±0.0171	0.1533±0.0116	0.1575±0.0202
Epididymis	0.0772±0.0022	0.0763±0.0043	0.0742±0.0042	0.0739±0.0094
Seminal vesicle	0.4440±0.0481	0.4520±0.0231	0.4372±0.0470	0.4215±0.0686
prostate	0.0134±0.0050	0.0133±0.0025	0.0124±0.0021	0.0150±0.0022

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 3. Absolute organ weights (g) of F_1 male mice born of F_0 male treated with diethanolamine on PND 21

Treatment (mg/kg)	control	20	80	320
Final Body Weight	10.22±1.33	10.32±1.03	9.90±0.79	10.11±0.78
Brain	0.3845±0.0167	0.3785±0.0232	0.3657±0.0305	0.3761±0.0320
Liver	0.5761±0.1111	0.4996±0.0841	0.5083±0.0827	0.4938±0.0530
Kidney	0.1549±0.0229	0.1476±0.0171	0.1452±0.0149	0.1446±0.0130
Thymus	0.0685±0.0101	0.0632±0.0066	0.0641±0.0083	0.0697±0.0112
Spleen	0.0797±0.0115	0.0743±0.0104	0.0748±0.0065	0.0849±0.0166
Adrenal gland	0.0026±0.0004	0.0024±0.0008	0.0026±0.0005	0.0024±0.0005
Testis	0.0491±0.0063	0.0465±0.0089	0.0440±0.0048	0.0487±0.0051
Epididymis	0.0121±0.0007	0.0114±0.0024	0.0096±0.0012*	0.0109±0.0028*
Seminal v. & prostate	0.0079±0.0022	0.0066±0.0013	0.0062±0.0008	0.0062±0.0016

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 4. Absolute organ weights (g) of F_1 female mice born of F_0 male treated with diethanolamine on PND 21

Treatment (mg/kg)	control	20	80	320
Final Body Weight	9.40±1.14	10.40±0.92	9.96±1.26	9.61±0.81
Brain	0.3751±0.0208	0.3531±0.0875	0.3895±0.0214	0.3839±0.0286
Liver	0.4649±0.0712	0.5382±0.0873	0.4935±0.0936	0.4547±0.0704
Kidney	0.1437±0.0185	0.1570±0.0178	0.1468±0.0213	0.1369±0.0109
Thymus	0.0637±0.0135	0.0644±0.0090	0.0648±0.0117	0.0680±0.0082
Spleen	0.0729±0.0139	0.0785±0.0082	0.0838±0.0082	0.0793±0.0187
Adrenal gland	0.0032±0.0013	0.0026±0.0008	0.0031±0.0015	0.0029±0.0010
Uterus	0.0064±0.0019	0.0084±0.0023	0.0070±0.0015	0.0091±0.0029
Ovary	0.0032±0.0004	0.0035±0.0010	0.0024±0.0008	0.0035±0.0012

Mean±SD

Table 5. Absolute organ weights (g) of F_1 male mice born of F_0 male treated with diethanolamine on PND 70

Treatment (mg/kg)	control	20	80	320
Final Body Weight	24.48±2.20	23.50±1.82	25.35±2.17	23.49±1.65
Brain	0.4045±0.0359	0.4253±0.0192	0.4221±0.0224	0.4195±0.0181
Liver	1.1811±0.1847	1.0342±0.1863	1.1582±0.1325	1.0481±0.1100
Kidney	0.3096±0.0421	0.3161±0.0270	0.3230±0.0286	0.3045±0.0219
Thymus	0.0434±0.0042	0.0449±0.0046	0.0407±0.0044	0.0386±0.0051
Spleen	0.0581±0.0028	0.0597±0.0074	0.0585±0.0069	0.0562±0.0061
Adrenal gland	0.0033±0.0008	0.0031±0.0006	0.0034±0.0009	0.0031±0.0005
Testis	0.1637±0.0097	0.1674±0.0114	0.1611±0.0175	0.1446±0.0262*
Epididymis	0.0632±0.0053	0.0632±0.0036	0.0641±0.0029	0.0581±0.0057*
Seminal vesicle	0.2246±0.0385	0.2065±0.0309	0.2444±0.0161	0.2245±0.0148
prostate	0.0101±0.0015	0.0088±0.0018	0.0078±0.0019*	0.0085±0.0010

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 6. Absolute organ weights (g) of F_1 female mice born of F_0 male treated with diethanolamine on PND 70

Treatment (mg/kg)	control	20	80	320
Final Body Weight	21.37±1.58	21.79±1.87	19.63±1.62	20.29±2.64
Brain	0.4387±0.0257	0.4601±0.0134	0.4435±0.0266	0.4473±0.0179
Liver	1.1251±0.1599	1.2376±0.1525	1.1374±0.1365	1.2188±0.2901
Kidney	0.2859±0.0286	0.2855±0.0257	0.2721±0.0287	0.2760±0.0447
Thymus	0.0642±0.0046	0.0606±0.0081	0.0578±0.0068	0.0566±0.0105
Spleen	0.0730±0.0085	0.0756±0.0093	0.0716±0.0068	0.0679±0.0114
Adrenal gland	0.0054±0.0008	0.0059±0.0014	0.0060±0.0011	0.0061±0.0012
Uterus	0.0633±0.0145	0.0784±0.0401	0.0794±0.0217	0.0736±0.0301
Ovary	0.0065±0.0011	0.0068±0.0017	0.0071±0.0010	0.0065±0.0014

Mean±SD

Table 7. Physical development and reflex of F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)	control	20	80	320
Sensory hair	4.0±0	4.0±0	4.0±0	4.0±0
Pinna detachment	4.0±0	4.0±0	4.0±0	4.0±0
Visible pilation	7.0±0	7.0±0	7.4±1.21	9.1±6.61
Pinna reflex	7.0±0	7.0±0	7.0±0	7.0±0
Surface righting reflex	9.0±0	9.0±0	9.0±0	9.0±0
Negative geotaxis	9.0±0	9.0±0	9.2±0.60	9.0±0
Incisor eruption	10.0±0	10.0±0	10.0±0	10.0±0
Growth of claw	10.0±0	10.0±0	10.0±0	10.0±0
Midair righting reflex	14.0±0	14.0±0	14.0±0	14.0±0
Cliff avoidance	14.0±0	14.0±0	14.0±0	14.0±0
Wire grasping	14.2±0.63	14.0±0	14.1±0.30	14.0±0
Eye opening	14.0±0	14.0±0	14.0±0	14.0±0
Cornea reflex	20.0±0	20.0±0	20.0±0	20.0±0
Testis descent	21.5±4.14	21.3±0.50	23.1±1.30	24.2±2.71
Vaginal opening	30.0±0	30.0±0	31.8±3.92	30.1±0.30

Mean±SD

Table 8. Hot plate test of F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)		Control	20	80	320
Hot plate	male	22.22±2.514	31.39±7.208*	28.69±4.516*	28.88±4.100*
	female	16.18±4.605	20.37±7.364	26.97±4.451*	30.64±6.074*

Mean±SD (*Significantly different from the control, $p<0.05$)

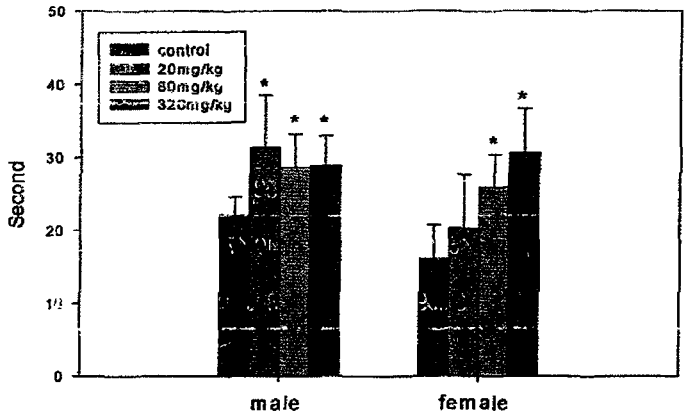

Figure 3. Hot plate test of F_1 mice born of F_0 male treated with diethanolamine. (*Significantly different from the control, $p<0.05$)

Table 9. Rota rod test of F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)	Control	20	80	320
male	1.05±0.517	0.68±0.474	1.16±0.620	1.13±0.625
female	1.45±0.584	1.03±0.521	0.99±0.447	1.03±0.416

Mean±SD

Table 10. Open field test of F_1 mice born of F_0 male treated with diethanolamine

Sex	Measurement	Treatment(mg/kg)			
		Control	20	80	320
	Total Distance(mm)	3480.5±888.2	3402.5±711.6	4027.2±779.5	3386.1±498.0
Male	V max	58.32±58.911	33.74±19.262	67.81±50.276	80.23±68.580
	V mean	6.16±0.999	5.68±1.171	6.93±1.415	6.40±1.793
	Total Distance(mm)	3560.2±331.3	3535.7±517.1	3722.9±477.8	3496.9±479.6
Female	V max	28.78±4.481	38.20±27.505	31.61±7.085	28.26±1.931
	V mean	5.93±0.524	6.29±0.962	6.54±0.847	6.06±0.806

Mean±SD

Table 11. Fear conditioning test of F_1 mice born of F_0 male treated with diethanolamine

Sex	Measurement	Treatment (mg/kg)			
		Control	20	80	320
Male	Context	55.11±21.526	40.64±14.962	43.54±14.896	46.67±23.942
	Cs	54.51±21.747	51.22±21.168	52.17±26.215	46.67±20.456
Female	Context	56.29±21.819	46.01±13.738	40.47±14.776	49.81±24.078
	Cs	34.07±5.340	34.99±2.994	34.30±11.525	34.06±4.595

Mean±SD

Table 12. Passive avoidance test of F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)	Control	20	80	320
male	33.88±15.487	28.00±12.293	27.38±7.744	25.56±12.817
female	29.56±16.164	13.00±9.250	24.20±18.079	29.75±25.348

Mean±SD

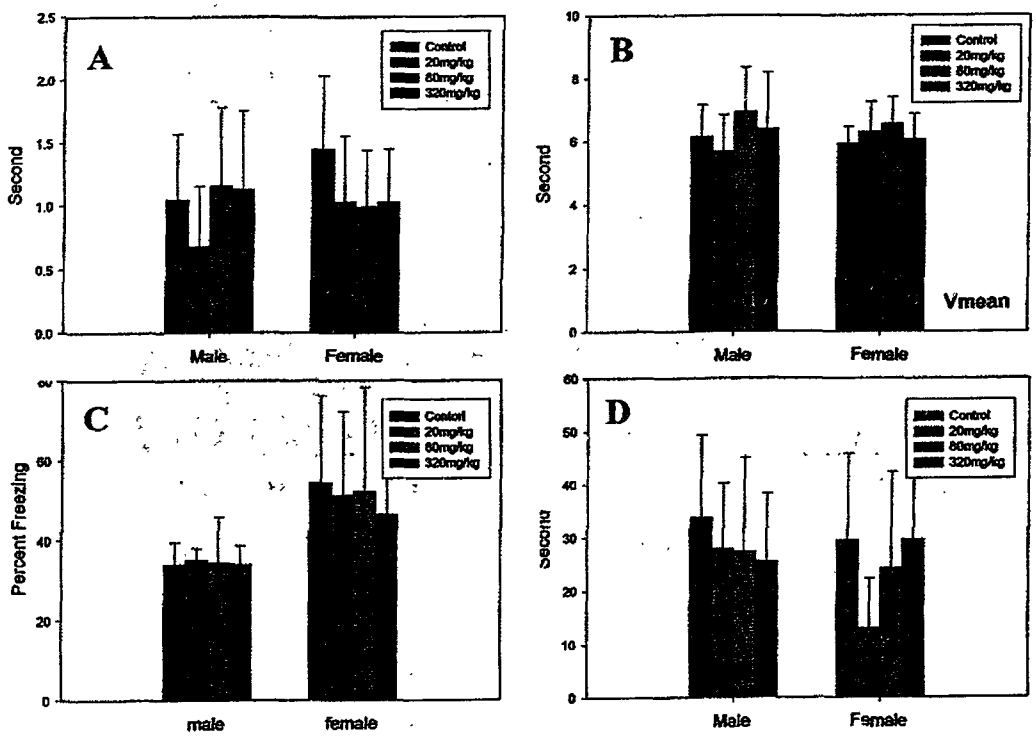

Figure 4. Rota-rod (A), open field (B), fear conditioning (C) and passive avoidance (D) test of F_1 mice born of F_0 male treated with diethanolamine

Table 13. Sperm motility and other parameter of sperm in F_0 male mice treated with diethanolamine

Parameter \ Treatment (mg/kg)	control	20	80	320
Sperm motility (%)	76.90±7.88	63.00±9.85	62.40±9.74	59.80±11.27
Path velocity (㎛/s)	39.1±1.9	35.5±2.5	34.7±2.6	34.4±2.7
Prog. Velocity (㎛/s)	24.5±2.1	21.3±2.9	21.2±3.1	21.4±3.3
Track speed (㎛/s)	81.6±3.4	75.4±3.2	73.1±4.0	72.6±4.4
Lateral amplitude(㎛/s)	6.5±0.4	6.2±0.7	6.0±0.7	5.6±0.8
Beat frequency(Hz)	31.8±1.5	32.3±2.2	32.6±2.2	32.8±1.5
Straightness(%)	59.8±4.0	57.4±4.5	57.7±6.5	58.6±5.7
Linearity(%)	30.7±2.4	29.3±2.8	29.8±4.3	30.4±3.6
Elongation(%)	50.5±4.7	44.8±14.3	50.2±4.3	47.1±4.5

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 14. Sperm motility and other parameter of sperm in F_1 mice born of F_0 male treated with diethanolamine

Parameter \ Treatment (mg/kg)	control	20	80	320
Sperm motility (%)	73.30±6.38	69.20±9.65	66.90±4.36	57.80±13.54
Path velocity (㎛/s)	39.2±3.1	36.0±1.8	34.6±2.3	33.8±2.9
Prog. Velocity (㎛/s)	23.4±2.7	19.2±6.7	21.2±2.8	20.1±4.0
Track speed (㎛/s)	82.7±4.9	77.2±3.3	75.2±3.6	72.6±4.2
Lateral amplitude(㎛/s)	6.7±0.5	6.7±0.6	6.3±0.6	5.8±0.7
Beat frequency(Hz)	33.1±1.7	33.6±1.3	33.9±1.2	34.0±1.8
Straightness(%)	55.3±4.4	56.0±3.8	58.1±5.5	56.1±7.2
Linearity(%)	28.2±2.7	28.1±1.9	29.0±3.0	28.5±4.8
Elongation(%)	46.3±1.8	45.3±2.3	45.1±3.2	47.3±5.5

Mean±SD (*Significantly different from the control, $p<0.05$)

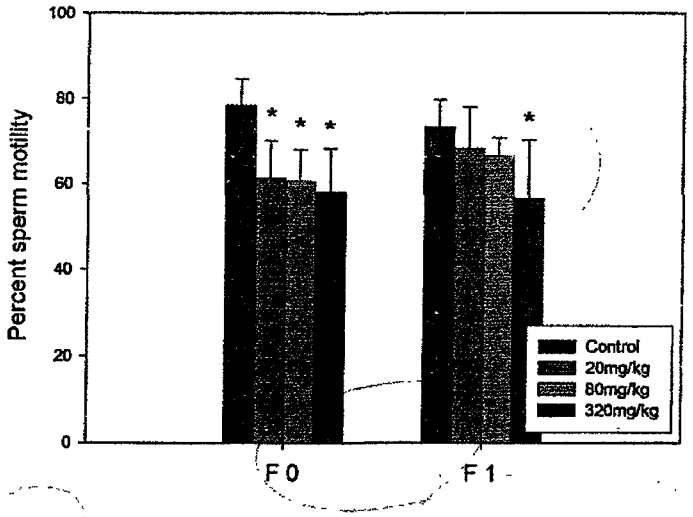

Figure 5. Sperm motility and other parameter of sperm in F_0 male mice treated with diethanolamine and F_1 mice born of F_0 male treated with diethanolamine. (*Significantly different from the control, $p<0.05$)

Table 15. Body weight of F_1 generation mice born of F_0 female treated with diethanolamine

Treatment (mg/kg)		Control	20	80	320
PND4	Male	2.84±0.19	2.75±0.45	2.92±0.30	2.45±0.39
	Female	2.90±0.35	2.72±0.50	2.80±0.42	2.47±0.31
PND7	Male	4.49±0.27	4.10±0.73	4.26±0.20	3.65±0.54*
	Female	4.66±0.50	3.88±0.53*	4.15±0.29	3.79±0.27*
PND14	Male	7.31±1.24	6.31±1.53	6.59±1.28	6.16±0.63
	Female	7.35±0.65	6.11±1.37*	6.34±0.63	6.19±0.32*
PND21	Male	10.16±0.92	9.66±1.38	9.48±0.77	8.21±1.08*
	Female	10.13±1.33	9.01±0.64	9.12±0.73	8.18±0.42*
PND28	Male	15.65±0.71	15.58±1.37	15.10±0.94	12.94±1.60*
	Female	13.54±0.52	13.04±0.34	13.64±1.17	12.06±0.54*
PND35	Male	18.68±1.10	19.14±1.68	18.93±0.90	17.44±1.13
	Female	16.73±0.80	15.89±0.80	16.29±1.15	15.41±0.40
PND42	Male	20.00±0.80	20.61±1.63	20.30±1.08	19.24±1.14
	Female	18.10±1.02	17.00±0.90	17.39±0.95	16.48±0.49*
PND49	Male	21.20±1.42	21.64±1.68	21.70±1.02	21.05±1.18
	Female	19.33±1.10	18.07±1.09	18.45±1.23	17.44±0.41*
PND56	Male	22.27±1.69	22.92±1.68	23.14±1.25	22.99±1.33
	Female	19.75±1.03	18.74±0.70	19.37±1.34	18.28±0.59
PND63	Male	23.40±1.71	24.21±1.85	24.53±1.25	23.95±1.25
	Female	20.48±1.14	19.27±0.69	20.10±1.61	18.89±0.33
PND70	Male	24.01±1.51	24.83±1.73	25.33±1.24	24.07±1.46
	Female	21.07±1.67	19.72±0.43	20.55±1.61	19.40±0.90

Mean±SD (*Significantly different from the control, $p<0.05$)

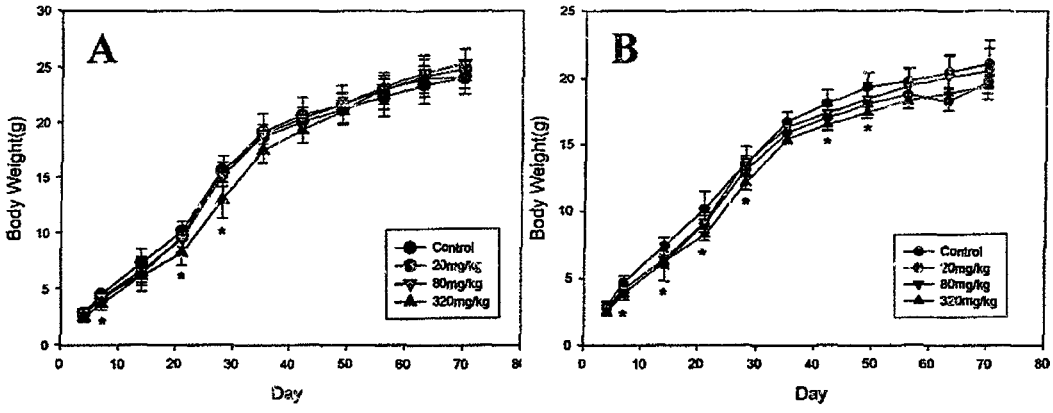

Figure 6. Change in body weight of F_1 male (A) and female (B) mice born of F_0 male treated with diethanolamine. (*Significantly different from the control, $p<0.05$)

Table 16. Absolute organ weights (g) of F_0 female mice treated with diethanolamine

Treatment (mg/kg)	Control	20	80	320
Final Body Weight	25.03±0.71	25.32±1.00	24.94±0.96	26.79±0.92
Brain	0.3899±0.0735	0.4243±0.0386	0.4188±0.0295	0.4344±0.0173
Liver	1.9773±0.1204	2.0082±0.1499	2.2711±0.1194	2.8276±0.3114
Kidney	0.3364±0.0107	0.3409±0.0224	0.3588±0.0224	0.4113±0.0316
Thymus	0.0534±0.0077	0.0495±0.0114	0.0462±0.0091	0.0573±0.0090
Spleen	0.0695±0.0037	0.0693±0.0043	0.0755±0.0066	0.0972±0.0231
Adrenal gland	0.0058±0.0011	0.0060±0.0011	0.0069±0.0011	0.0069±0.0011
Uterus	0.0649±0.0112	0.0606±0.0165	0.0840±0.0416	0.0578±0.0225
Ovary	0.0097±0.0034	0.0086±0.0020	0.0099±0.0011	0.0096±0.0021

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 17. Absolute organ weights (g) of F_1 male mice born of F_0 female treated with diethanolamine on PND 21

Treatment (mg/kg)	control	20	80	320
Final Body Weight	9.05±1.03	9.99±1.34	9.27±0.60	8.45±0.82
Brain	0.3763±0.0285	0.3781±0.0142	0.3781±0.0215	0.3714±0.0317
Liver	0.4796±0.0815	0.5063±0.0929	0.4958±0.0677	0.4189±0.0765
Kidney	0.1319±0.0162	0.1473±0.0227	0.1453±0.0125	0.1320±0.0140
Thymus	0.0543±0.0151	0.0663±0.0143	0.0675±0.0079	0.0639±0.0080
Spleen	0.0651±0.0121	0.0803±0.0081	0.0574±0.0061	0.0684±0.0112
Adrenal gland	0.0026±0.0009	0.0026±0.0007	0.0022±0.0006	0.0021±0.0009
Testis	0.0448±0.0154	0.0415±0.0098	0.0428±0.0039	0.0380±0.0063
Epididymis	0.0076±0.0018	0.0095±0.0020	0.0090±0.0013	0.0081±0.0022
Seminal v. & prostate	0.0052±0.0019	0.0065±0.0022	0.0052±0.0007	0.0045±0.0011

Mean±SD

Table 18. Absolute organ weights (g) of F_1 female mice born of F_0 female treated with diethanolamine on PND 21

Treatment (mg/kg)	control	20	80	320
Final Body Weight	9.40±1.07	9.27±1.06	9.45±0.90	8.54±0.52
Brain	0.3667±0.0327	0.3677±0.0290	0.3790±0.0328	0.3772±0.0233
Liver	0.5348±0.0292	0.4862±0.0548	0.5401±0.0842	0.4584±0.0573
Kidney	0.1456±0.0085	0.1525±0.0202	0.1516±0.0118	0.1447±0.0076
Thymus	0.0612±0.0102	0.0646±0.0076	0.0643±0.0085	0.0606±0.0090
Spleen	0.0650±0.0155	0.0655±0.0097	0.0703±0.0099	0.0558±0.0075
Adrenal gland	0.0024±0.0005	0.0024±0.0006	0.0024±0.0006	0.0021±0.0005
Uterus	0.0090±0.0026	0.0081±0.0018	0.0088±0.0023	0.0067±0.0008
Ovary	0.0034±0.0010	0.0028±0.0006	0.0033±0.0009	0.0027±0.0009

Mean±SD

Table 19. Absolute organ weights (g) of F_1 male mice born of F_0 female treated with diethanolamine on PND 70

Treatment (mg/kg)	control	20	80	320
Final Body Weight	28.87±2.42	29.52±3.46	31.50±2.80	26.59±2.32
Brain	0.4427±0.0168	0.4181±0.0224	0.4277±0.0282	0.4373±0.0617
Liver	1.3242±0.1668	1.2807±0.1646	1.3231±0.1031	1.2405±0.1263
Kidney	0.3707±0.0351	0.3429±0.0381	0.3493±0.0326	0.3382±0.0192
Thymus	0.0410±0.0040	0.0403±0.0046	0.0426±0.0067	0.0365±0.0033
Spleen	0.0634±0.0105	0.0638±0.0078	0.0591±0.0074	0.0607±0.0087
Adrenal gland	0.0040±0.0009	0.0033±0.0005*	0.0028±0.0005*	0.0036±0.0011
Testis	0.1628±0.0077	0.1661±0.0161	0.1659±0.0089	0.1573±0.0180
Epididymis	0.0702±0.0056	0.0706±0.0042	0.0725±0.0035	0.0672±0.0058
Seminal vesicle	0.3237±0.0385	0.3166±0.0442	0.3076±0.0318	0.3053±0.0411
prostate	0.0111±0.0027	0.0096±0.0027	0.0112±0.0020	0.0095±0.0021

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 20. Absolute organ weights (g) of F_1 female mice born of F_0 female treated with diethanolamine on PND 70

Treatment (mg/kg)	control	20	80	320
Final Body Weight	21.21±1.42	20.34±0.59	21.45±1.41	20.29±1.21
Brain	0.4419±0.0215	0.4256±0.0298	0.4306±0.0289	0.4171±0.0899
Liver	1.0596±0.0805	1.0367±0.0604	1.0740±0.0981	0.9745±0.2171
Kidney	0.2674±0.0166	0.2596±0.0158	0.2702±0.0199	0.2667±0.0157
Thymus	0.0651±0.0028	0.0605±0.0046	0.0606±0.0061	0.0580±0.0081
Spleen	0.0660±0.0046	0.0682±0.0042	0.0729±0.0082	0.0687±0.0079
Adrenal gland	0.0065±0.0011	0.0056±0.0006	0.0059±0.0010	0.0062±0.0009
Uterus	0.1206±0.0505	0.1061±0.0493	0.0824±0.0278*	0.0700±0.0184*
Ovary	0.0074±0.0007	0.0069±0.0011	0.0076±0.0016	0.0073±0.0015

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 21. Physical development and reflex of F_1 mice born of F_0 female treated with diethanolamine

Treatment (mg/kg)	control	20	80	320
Sensory hair	4.0±0	4.0±0	4.0±0	4.0±0
Pinna detachment	4.0±0	4.0±0	4.0±0	4.0±0
Visible pilation	7.0±0	7.0±0	7.0±0	7.0±0
Pinna reflex	7.0±0	7.0±0	7.0±0	7.0±0
Surface righting reflex	9.0±0	9.0±0	9.0±0	9.0±0
Negative geotaxis	9.0±0	9.0±0	9.0±0	9.0±0
Incisor eruption	10.0±0	10.0±0	10.0±0	10.0±0
Growth of claw	10.0±0	10.0±0	10.0±0	10.0±0
Midair righting reflex	14.0±0	14.0±0	14.0±0	14.0±0
Cliff avoidance	14.0±0	14.0±0	14.0±0	14.0±0
Wire grasping	14.0±0	14.0±0	14.0±0	14.0±0
Eye opening	14.0±0	14.0±0	14.0±0	14.0±0
Cornea reflex	20.0±0	20.0±0	20.0±0	20.0±0
Testis descent	21.4±1.34	22.8±1.64	23.4±0.88	24.7±3.64
Vaginal opening	30.8±0.45	30.8±0.45	31.4±1.88	31.9±0.90

Mean±SD

Table 22. Hot plate test of F_1 mice born of F_0 female treated with diethanolamine

Treatment (mg/kg)		Control	20	80	320
Hot plate	male	19.84±6.195	33.35±19.253	25.03±3.151	29.89±5.743
	female	18.29±3.185	24.89±6.287	25.79±6.289	28.99±9.704

Mean±SD

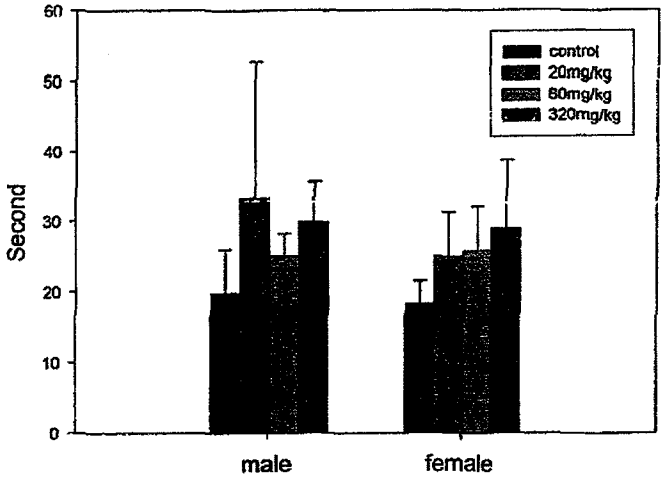

Figure 7. Hot plate test of F_1 mice born of F_0 female treated with diethanolamine

Table 24. Open field test of F_1 mice born of F_0 female treated with diethanolamine

Sex	Measurement	Treatment (mg/kg)			
		Control	20	80	320
Male	Total Distance(mm)	2890.4±496.4	4043.2±1810.2	3282.8±1024.6	3148.3±1231.6
	V max	28.38±5.575	50.93±29.442*	29.49±4.711	26.13±4.974
	V mean	4.81±0.827	8.21±3.478	6.51±3.705	5.89±2.810
Female	Total Distance(mm)	3357.7±596.4	2917.3±1066.6	3217.5±847.2	4173.3±627.2
	V max	27.32±1.943	29.17±6.200	29.23±3.202	31.6[illegible]±3.969
	V mean	5.58±1.006	5.44±1.246	5.63±0.979	6.95±[illegible]

Mean±SD

Table 25. Fear conditioning test of F_1 mice born of F_0 female treated with diethanolamine

Sex	Measurement	Treatment (mg/kg)			
		Control	20	80	320
Male	Context	34.73±5.827	36.10±9.713	33.67±3.280	35.56±9.737
	Cs	39.02±14.507	29.55±5.251	29.79±5.869	29.98±5.023
Female	Context	69.43±11.549	60.67±19.662	63.88±18.015	65.70±17.700
	Cs	35.17±12.090	32.58±4.216	31.59±3.836	32.50±3.964

Mean±SD

Table 26. Passive avoidance test of F_1 mice born of F_0 female treated with diethanolamine

Treatment (mg/kg)	Control	20	80	320
male	35.75±19.293	26.63±11.747	22.50±10.515	27.44±11.717
female	54.71±69.980	28.71±18.590	27.22±14.646	37.25±21.486

Mean±SD

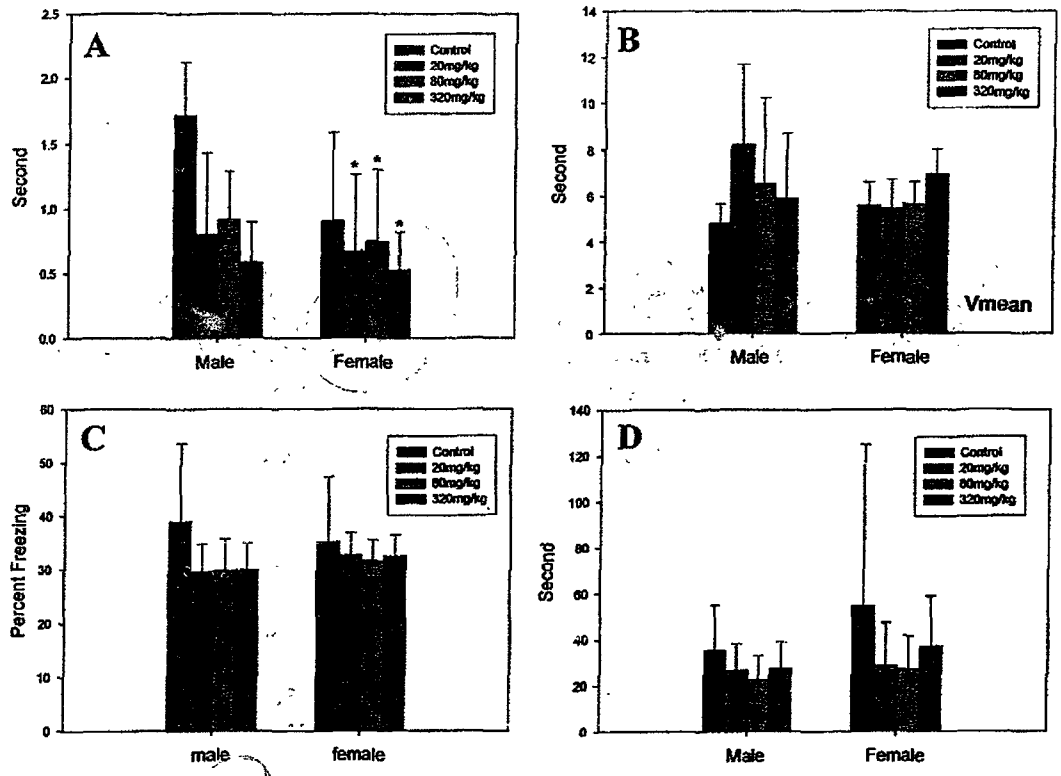

Figure 8. Rota-rod (A), open field (B), fear conditioning (C) and passive avoidance (D) test of F_1 mice born of F_0 female treated with diethanolamine

Table 27. Sperm motility and other parameter of sperm in F_1 mice born of F_0 female treated with diethanolamine

Parameter \ Treatment (mg/kg)	control	20	80	320
Sperm motility (%)	70.60±11.81	65.50±7.89	64.60±7.14	67.80±8.97
Path velocity (㎛/s)	37.8±4.3	35.0±1.9	37.3±1.7	36.8±2.0
Prog. Velocity (㎛/s)	23.5±3.6	21.3±2.3	23.4±2.4	21.7±1.8
Track speed (㎛/s)	78.9±7.0	75.1±2.9	77.4±2.5	76.4±4.2
Lateral amplitude(㎛/s)	6.1±0.8	6.1±0.5	6.4±0.7	6.5±0.8
Beat frequency(Hz)	32.0±1.6	33.2±1.1	31.6±2.0	32.2±1.2
Straightness(%)	56.9±8.7	58.1±4.2	59.8±4.3	55.2±4.4
Linearity(%)	30.4±2.4	29.6±2.5	31.0±2.7	28.8±2.3
Elongation(%)	49.8±3.2	44.8±2.2	45.6±2.3	46.7±4.0

Mean±SD

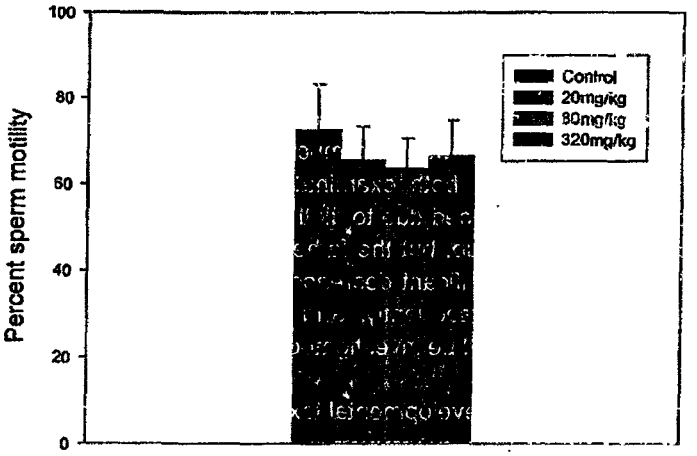

Figure 9. Sperm motility and other parameter of sperm in F_1 mice born of F_0 female treated with diethanolamine

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A Study on the Reproductive and Developmental Toxicity of Diethanolamine

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Abstract

Diethanolamine (DEA) is used as an emulsifier, moistening agent, surfactant as well as a thickening agent in products used for various household needs. In other words, Diethanolamine is used in such products as shampoo, hair conditioner, soap, sun block, cosmetics, hair spray, and perm (hair coloring) agents, and also in medicines as well as in agricultural chemicals. Diethanolamine was noticed to be a carcinogenic substance as it can be easily absorbed and may then cause stomach cancer, esophageal cancer, liver cancer, and bladder cancer, among others. There are recent reports indicating its harmful effects on fetal brain development, leading to memory and learning disorders when a pregnant woman is exposed to the substance. The possibility of malformation, as diethanolamine effects spermatogenesis in males, is also being studied. However, to date, no detailed experimental results on the effects on spermatogenesis toxicity and on future generations have been reported. The purpose of this study is to clarify the harmful effects of diethanolamine from exposure to the fathers' and mothers' bodies, which are highly likely to be exposed in large amounts through products for general household needs. In order to examine the effects due to exposure to the father's body, diethanolamine was applied to male mice for 4 weeks (0, 20, 80, 320mg/kg). They were caused to copulate with normal females for fetuses. For experimenting effects due to exposure to mothers' bodies, diethanolamine was applied during pregnancy and the organ weight, fertility, body growth, reflex functions, movement, learning, and memory abilities of the fetuses were evaluated. Results showed that the epididymis weight decreased significantly in fetuses born after exposure of diethanolamine to the father. These fetuses also had a very significantly slow response to pain compared with the solvent control group. Among them, the weight increase rate of female fetuses was meaningfully reduced and the movement power was also much lower than the solvent control group. Movement, learning, and memory abilities in both examinations of exposure to the fathers' and mothers' bodies appeared to be generally reduced due to diethanolamine. Sperm motility was slightly decreased in the mother's body exposure group, but the fathers and fetuses born of a father's body subject to exposure showed a remarkably significant decrease. Studies of diethanolamine effects on future generations should be expanded subsequently, and the mechanism of reproductive and developmental toxicity of diethanolamine should be investigated further.

Keywords: diethanolamine, reproductive and developmental toxicity, and effects on future generations

Introduction

Diethanolamine (DEA) is used as an emulsifier, moistening agent, surfactant as well as thickening agent in products used for various household needs, i.e., it is used as an in shampoo, hair conditioner, soap, sun block, cosmetics, hair spray, and perm (hair coloring) and also in medicines and agricultural chemicals. Over 100 thousand tons of the substance is being used every year in the U.S.

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Diethanolamine was noticed to be a carcinogenic substance as it can be easily absorbed and thus may cause stomach cancer, esophageal cancer, liver cancer, and bladder cancer, etc. Some professionals pointed out that diethanolamine takes up 25% of the total amount of chemicals contained especially in shampoo products for generating more foam. However, information on the harmful effects of reproductive and developmental toxicity is extremely limited. There are recent reports

indicating its harmful effects on the fetal brain development process leading to memory and learning disorders when a pregnant woman is exposed to the substance. The possibility of malformation as it participates in spermatogenesis of males is also being studied. Nonetheless, no detailed results from on the effects on spermatogenesis toxicity and on future generations have thus far been reported. Therefore, an in vivo study that appropriately combines overall assessment on harmful diethanolamine effects on the parents' generation, which has a great exposure risk since it is contained in general household products, and the standard test method of reproductive and developmental toxicity was conducted in this study. Our purpose was to contribute to clarifying the danger of diethanolamine based on study results and to protect the health of future generations.

Materials and Methods

In order to examine the reproductive and developmental toxicity of diethanolamine, experiments were run separately on the toxicity on fetuses from the father's body (male) exposure and on those from the mother's body (female) exposure.

1. Reproductive and developmental toxicity test

A. Father's body exposure

The fur in the scapula region was removed with an animal hair remover in 5 week-old C57BL/6 male fetuses. Then 0, 20, 80, and 320 mg/kg of diethanolamine were applied to the skin of 15 fetuses in each group at the same time in the morning everyday for 4 weeks. The treatment group that received diethanolamine application for 4 weeks and the control group were kept together with 9 week-old C57BL/6 female fetuses at a ratio of 1:1. A vaginal plug test was done the next day. It was accepted as gestational day 0 (GD 0) if a vaginal plug was confirmed.

B. Mother's body exposure

9 week-old C57BL/6 female and male fetuses were kept together at 1:1 and a vaginal plug test was done the next day. It was accepted as GD 0 if a vaginal plug was confirmed, and pregnant mothers' bodies were prepared. At GD 6, an animal hair remover was used to remove fur in the scapular region. 0, 20, 80, and 320 mg/kg of diethanolamine were applied to the skin of 10 fetuses of each group at the same time in the morning everyday for 4 weeks. The delivery day was accepted as postnatal day 0 (PND 0) and diethanolamine was applied to the mother's body until PND 21. The number of fetuses was adjusted to 4 males and 4 females on PND 4. After weaning, an autopsy was done on the mother's body and fetuses (two females and two males each). The remaining fetuses were reared continuously in order to examine their behavior and development.

C. Common items

Specific pathogen free (SPF) C57BL/6 female and male fetuses were purchased from Orient Co., Seoul, Korea. A clean breeding facility of the National Institute of Toxicological Research, KFDA which received certification from the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) was used. Products of diethanolamine from Sigma (St. Louis, MO, U.S.A. D0681, 98.5%) and ethanol from Merck (Germany) were chosen for running the experiment. Diethanolamine of 20, 80, 320 mg/kg with ethanol as the solvent were applied to 2 cm^2 between the scapulas at the same time every morning. Ethanol was applied to the same region of the control group. Fur on the application region between the scapulas was removed with an animal hair remover. (Figure 1)

The weight of each female and male fetus (F1) was measured on PND 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70. The amount of feed consumed by the father, mother, and fetuses was measured once a week in order to examine the gains. Female and male fetuses were kept together at 1:1 and a vaginal plug test was done the next day. It was accepted as GD 0 if a vaginal plug was confirmed. The final assessment of pregnancy depended on the existence of a fetus within the uterus at autopsy, marks of implantation, or delivery of fetus by normal delivery. Autopsies were done on exposed fathers' bodies and exposed mothers' bodies during pregnancy. Skeletal abnormalities of fetuses at GD 18 were evaluated. The remaining fetuses were weaned from the mother at PND 21 and autopsied.

Figure 1. Dermal application

D. Evaluation of fetuses

General symptoms and deaths of the entire experiment group were observed once a day during the test period. If an abnormality was discovered, the type of symptom, date and time of discovery, and its duration were recorded. The severity of the symptom was also recorded if necessary.

The number of fetuses per mother's body was adjusted to 4 females and 4 males, 8 fetuses in total on PND 4 in order to examine the growth and development. The number of fetuses at birth and after adjustment was compared and recorded. The fetuses were weaned from the mother on PND 21 and autopsies were done on 2 female and 2 male fetuses each per mother's body. General organs and reproductive organs were compared. Gross lesions were fixed and preserved in 10% neutral formalin.

After weaning autopsy, tests were performed on the remaining fetuses to confirm the growth, development, and behavior - sensory hair and pinna detachment on PND 4; visible pilation and reflex on PND 7; surface righting reflex and negative geotaxis on PND 9; incisor eruption and growth of claw (hind leg) on PND 10; midair righting reflex on PND 14; cliff avoidance, wire grasping and eye opening on PND 14; corneal reflex and testis descent on PND 20; rota rod test on PND 28; vaginal opening on PND 30; open field on PND 35; passive avoidance test on PND 42; hot plate test on PND 45; and fear conditioning test on PND 63.

The weight of general organs and reproductive organs of mature female and male fetuses (PND 70) that completed all tests on growth, development, and behavior were measured. Organs and gross lesions were fixed and preserved in 10% neutral formalin.

E. Measurement of sperm motility

The sperm motility and velocity of fathers' bodies exposed to DEA and male fetuses produced from exposure of the father and mother bodies were measured by using the Hamilton-Thorne IVOS 2000 system.

F. Statistical interpretation

After the ANOVA test, the Dunnett's test was used to examine the significance between the solvent control group and the group that received the testing substance administration by $p<0.05$.

Results and Conclusions

A. Evaluation of toxicity on fetuses from father body exposure

Clinical symptoms due to the injection of the testing substance were not observed in all administered groups while applying diethanolamine (0, 20, 80, 320 mg/kg) on the scapular region for 4 weeks. There were no animal deaths. Keratinization was found on the area applied with high-dosage but said area soon recovered.

As for the father body, there was no difference found in weight changes of fetuses between the solvent control group and testing substance administered group during the 4 weeks of diethanolamine application, but a similar increase rate occurred. Among the female and male fetuses born of the fathers' bodies exposed to diethanolamine, the weight of female fetuses was not different from the solvent control group. The weight of male fetuses in the high-dosage group (320 mg/kg) was slightly lower than those of the solvent control group but no significance was observed. (Table 1, Figure 2)

Results of autopsies done after administering diethanolamine for 4 weeks showed no abnormal manifestations in the organs of the animals in the solvent control group or in the testing substance administered group.

Main organs such as the brain, liver, spleen and kidneys along with reproductive organs like the testis and epididymis were extracted and their weights were measured. The epididymis weight showed a slight decrease in higher dosages when compared with the solvent control group, but the difference was not significant. (Table 2) Female and male fetuses were autopsied at the weaning period (PND 21) and at maturity (PND 70). Their general organs and reproductive organs were extracted and weights were measured. According to results, the epididymis weight of male fetuses from the

weaning period autopsy at diethanolamine of 80 mg/kg was significantly decreased, while the uterus weight of female fetuses was increased. (Table 3, 4) Autopsy results at maturity indicated a meaningful reduction in male reproductive organ weight in the group of maximum dosage 320 mg/kg. Females also showed a similar feature with the weaning period autopsy, having a heavier uterus. (Table 5, 6)

Test results of the intestines and skeletal abnormalities of survived fetuses that were extracted by Caesarean operation on GD 18 showed no significant difference between the solvent control group and diethanolamine administered group. In order to examine the growth, development, and behavior of female and male fetuses, an evaluation on physical development (sensory hair, pinna detachment, eye opening, incisor eruption, growth of claw, testis descent, vaginal opening, etc.), reflex functions (pinna reflex, corneal reflex), and posture control functions (righting reflex, negative geotaxis) was done by each period. Although no significance was found in the high-dosage group compared with the solvent control group, visible pilation lagged and negative geotaxis decreased. Testis descent which reflects sexual maturity of males was also slower in the high-dosage group than the solvent control group (Table 7). Hot-plate test results showed a meaningful dosage-dependent increase than in the control group (Table 8 & Figure 3).

When measuring the movement ability (rota rod test) of female and male fetuses, only the 20mg/kg group among males had a slight decrease and the high-dosage group among females appeared to have a decreasing tendency. However, the results were not significant, (Table 9 & Figure 4) As for results of open field which measures the amount of movement within a fixed space per hour, both male and female groups were similar to those of the control group. (Table 10 & Figure 4) The fear conditioning test which assesses the cognitive ability by exposing subjects to noise and electrical shock showed a decrease in both male and female groups when compared with the solvent control group, but the difference was not significant. (Table 11 & Figure 4) Results of the passive avoidance test which examines the ability to recognize the situation by administering electrical shocks also appeared to have an insignificant decrease in both groups. (Table 12 & Figure 4)

The sperm motility and velocity of the epididymis were examined and compared with the solvent control group in order to study how diethanolamine affects the reproductive function of male fetuses through the father's body. As for fathers' bodies, the sperm motility was significantly, dosage-dependently decreased than the control group. Fetuses also showed a dosage-dependent decrease. The results in the high-dosage group of 320 mg/kg were especially meaningful. (Table 13, 14 & Figure 5).

B. Evaluation of toxicity on fetuses from the mother's body exposure

Clinical symptoms due to the injection of the testing substance were not observed in all administered groups while applying diethanolamine (0, 20, 80, 320 mg/kg) on the scapular region during pregnancy and lactation period. There were no animal deaths. Keratinization occurred at the area applied with high-dosage but said area soon recovered.

As for the weight change of pregnant mothers' bodies while being exposed to diethanolamine, there was no difference found between the solvent control group and the testing substance administered group. Rather, they both showed a similar rate of increase. The weight of female and male fetuses born of mother bodies that were exposed to diethanolamine during pregnancy was significantly decreased than in the solvent control group. Especially, the high-dosage female fetus group weighed meaningfully less from PND 7 to PND 49 than the control group. (Table 15 & Figure 6)

Autopsies done after administering diethanolamine during pregnancy and lactation period showed no abnormal manifestations in the organs of the animal in both the solvent control group and testing substance administered group.

Main organs such as the brain, liver, spleen and kidneys along with reproductive organs like the testis and epididymis were extracted and their weights were measured. The uterus weight in the 80 mg/kg dosage group was higher than the control group but was not significant. General organs were practically slightly heavier. (Table 16) Female and male fetuses were autopsied at the weaning period (PND 21) and at maturity (PND 70). Their general organs and reproductive organs were extracted and weights were measured. According to the results, both female and male fetuses did not show any difference from the control group. No specific finding was noticed in the results of organ weights from the autopsy at female fetus maturity. Only the adrenal weight of the administered group was found to be significantly decreased from the autopsy done at male fetus maturity. (Table 17-20).

Test results of the intestines and skeletal abnormalities of survived fetuses that were extracted by Caesarean operation on GD 18 showed no significant difference between the solvent control group and the diethanolamine administered group. However, an observation experiment is currently under way for an accurate interpretation.

In order to examine the growth, development, and behavior of female and male fetuses, an evaluation of physical development (sensory hair, pinna detachment, eye opening, incisor eruption, growth of claw, testis descent, vaginal opening, etc.), reflex functions (pinna reflex, corneal reflex), and posture control functions (righting reflex, negative geotaxis) was done at each period. Although no significance was found, testis descent which reflects sexual maturity of males was slower in the diethanolamine administered group than in the solvent control group (Table 21). Hot-plate test results showed a more dosage-dependent increase than in the control group (Table 22 & Figure 7).

As for the movement ability (rota rod test) of female and male fetuses, both males and females appeared to have a dosage-dependent decrease. Especially all the male administered group showed a meaningful reduction than in the control group. (Table 23 & Figure 8) Results of open field, which measures the amount of movement within a fixed space per hour, indicated that both male and female groups were similar to those of the control group. (Table 24 & Figure 8) The fear conditioning test, which assesses the cognitive ability by exposing subjects to noise and electrical shocks, showed a decrease in both male and female groups when compared with the solvent control group, but the difference was not significant. (Table 25 & Figure 8) Results of the passive avoidance test, which examines the ability to recognize the situation by administering electrical shocks, also appeared to have an insignificant decrease in both groups. (Table 26 & Figure 8)

The sperm motility and velocity of the epididymis were examined and compared with the solvent control group in order to study the effects of diethanolamine on the reproductive functions of male fetuses, through the mothers' bodies exposed to diethanolamine during pregnancy and the lactation period. Although it was an insignificant result, the sperm motility seemed to be decreased. (Table 27 & Figure 9)

Male fetuses born of mother bodies exposed to diethanolamine during pregnancy lagged somewhat in testis descent and sperm motility. However, not only the fathers' bodies but also male fetuses born of the copulation of fathers exposed to diethanolamine and normal mothers showed a significant decrease in the epididymis weight. The high-dosage group of these fetuses also appeared with a meaningful decrease in sperm motility. This indicates that the father's body exposure affects spermatogenesis of future generations more than the mother's body exposure. Since sperm abnormalities may cause congenital malformations, more related studies are required.

Fetuses exposed to diethanolamine during pregnancy also showed a decline in physical growth, reflex functions, movement, learning, and memory abilities. Similar features also appeared as a result of the father's body exposure. This suggests that the father's body exposure can influence the future generations without the mother's body exposure. More related studies on this topic should be conducted in future.

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Table 1. Body weight of F_1 generation mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)		Control	20	80	320
PND4	Male	3.20±0.34	3.34±0.26	3.01±0.36	3.11±0.57
	Female	3.23±0.45	3.27±0.15	2.65±0.65	2.94±0.42
PND7	Male	4.67±0.53	5.19±0.27	4.36±0.59	4.71±0.35
	Female	4.72±0.67	5.00±0.13	4.18±0.68	4.78±0.27
PND14	Male	7.51±0.95	8.49±0.34	7.41±0.78	7.48±0.48
	Female	7.46±0.86	8.18±0.48	7.00±0.93	7.49±0.38
PND21	Male	11.06±1.13	11.21±0.47	10.16±1.01	10.00±1.88
	Female	10.81±1.11	10.82±0.58	9.51±1.34	10.70±0.75
PND28	Male	15.54±1.94	15.90±0.95	15.18±1.12	14.56±2.50
	Female	14.24±1.60	14.72±0.87	12.95±1.45	14.18±1.01
PND35	Male	18.64±1.47	19.20±1.33	18.85±0.61	17.90±2.47
	Female	16.62±1.24	16.69±1.58	15.80±1.34	16.51±1.28
PND42	Male	20.24±1.23	20.98±1.41	20.43±0.93	19.40±2.27
	Female	18.29±1.34	18.36±1.81	17.01±1.17	17.67±1.31
PND49	Male	21.08±1.56	21.49±1.60	21.42±1.01	19.23±3.78
	Female	18.76±1.49	18.76±1.61	17.55±1.20	18.20±1.49
PND56	Male	22.18±1.90	22.68±1.54	22.59±1.05	21.43±2.12
	Female	19.76±1.70	19.48±1.60	18.39±1.31	19.04±1.71
PND63	Male	23.27±1.55	23.50±1.48	23.52±1.17	22.03±2.36
	Female	20.41±1.77	20.22±1.81	18.95±1.23	19.72±2.02
PND70	Male	23.93±1.55	24.85±2.38	24.50±1.36	22.84±2.31
	Female	21.20±1.70	20.87±1.81	19.74±1.41	20.45±1.90

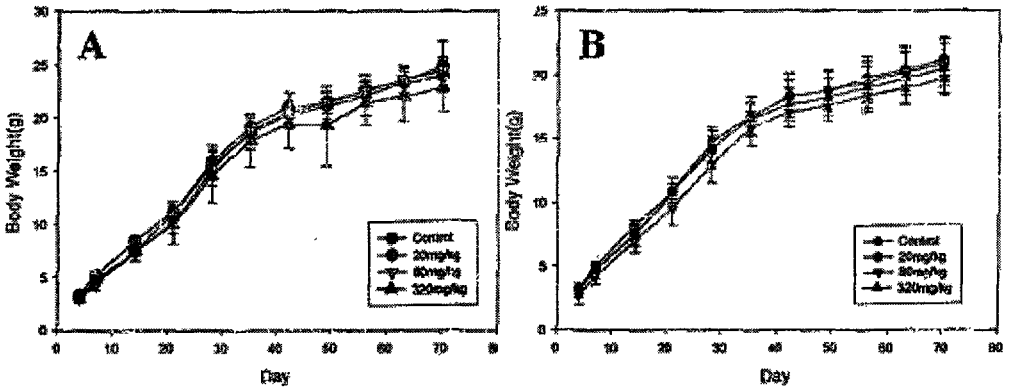

Figure 2. Change in body weight of F_1 male (A) and female (B) mice born of F_0 male treated with diethanolamine

Table 2. Absolute organ weights (g) of F_0 male mice treated with diethanolamine

Treatment (mg/kg)	control	20	80	320
Final Body Weight	34.10±3.34	35.17±2.51	33.88±4.19	33.36±3.25
Brain	0.4316±0.0120	0.4395±0.0189	0.4136±0.0339	0.4318±0.0319
Liver	1.4469±0.1281	1.4919±0.1354*	1.3765±0.1350	1.3897±0.1405
Kidney	0.3741±0.0202	0.4019±0.0227	0.3960±0.0337	0.3757±0.1136
Thymus	0.0346±0.0047	0.0363±0.0070	0.0365±0.0045	0.0372±0.0037
Spleen	0.0567±0.0087	0.0587±0.0066	0.0561±0.0206	0.0611±0.0079
Adrenal gland	0.0031±0.0006	0.0031±0.0006	0.0033±0.0009	0.0035±0.0004
Testis	0.1546±0.0072	0.1605±0.0171	0.1533±0.0116	0.1575±0.0202
Epididymis	0.0772±0.0022	0.0763±0.0043	0.0742±0.0042	0.0739±0.0094
Seminal vesicle	0.4440±0.0481	0.4520±0.0231	0.4372±0.0470	0.4215±0.0686
prostate	0.0134±0.0050	0.0133±0.0025	0.0124±0.0021	0.0150±0.0022

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 3. Absolute organ weights (g) of F_1 male mice born of F_0 male treated with diethanolamine on PND 21

Treatment (mg/kg)	control	20	80	320
Final Body Weight	10.22±1.33	10.32±1.03	9.90±0.79	10.11±0.78
Brain	0.3845±0.0167	0.3785±0.0232	0.3857±0.0305	0.3761±0.0320
Liver	0.5761±0.1111	0.4996±0.0841	0.5083±0.0827	0.4938±0.0530
Kidney	0.1549±0.0229	0.1476±0.0171	0.1452±0.0149	0.1446±0.0130
Thymus	0.0685±0.0101	0.0632±0.0066	0.0641±0.0083	0.0697±0.0112
Spleen	0.0797±0.0115	0.0743±0.0104	0.0748±0.0065	0.0849±0.0166
Adrenal gland	0.0026±0.0004	0.0024±0.0008	0.0026±0.0005	0.0024±0.0005
Testis	0.0491±0.0063	0.0465±0.0089	0.0440±0.0048	0.0487±0.0051
Epididymis	0.0121±0.0007	0.0114±0.0024	0.0096±0.0012	0.0109±0.0028*
Seminal v. & prostate	0.0079±0.0022	0.0066±0.0013	0.0062±0.0008	0.0062±0.0016

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 4. Absolute organ weights (g) of F_1 female mice born of F_0 male treated with diethanolamine on PND 21

Treatment (mg/kg)	control	20	80	320
Final Body Weight	9.40±1.14	10.40±0.92	9.96±1.26	9.61±0.81
Brain	0.3751±0.0208	0.3531±0.0875	0.3895±0.0214	0.3839±0.0286
Liver	0.4649±0.0712	0.5382±0.0873	0.4935±0.0936	0.4547±0.0704
Kidney	0.1437±0.0185	0.1570±0.0178	0.1468±0.0213	0.1369±0.0109
Thymus	0.0637±0.0135	0.0644±0.0090	0.0648±0.0117	0.0680±0.0082
Spleen	0.0729±0.0139	0.0785±0.0082	0.0838±0.0082	0.0793±0.0187
Adrenal gland	0.0032±0.0013	0.0026±0.0008	0.0031±0.0015	0.0029±0.0010
Uterus	0.0064±0.0019	0.0084±0.0023	0.0070±0.0015	0.0091±0.0029
Ovary	0.0032±0.0004	0.0035±0.0010	0.0024±0.0008	0.0035±0.0012

Mean±SD

Table 5. Absolute organ weights (g) of F_1 male mice born of F_0 male treated with diethanolamine on PND 70

Treatment (mg/kg)	control	20	80	320
Final Body Weight	24.48±2.20	23.50±1.82	25.35±2.17	23.49±1.65
Brain	0.4045±0.0359	0.4253±0.0192	0.4221±0.0224	0.4195±0.0181
Liver	1.1811±0.1847	1.0342±0.1863	1.1582±0.1325	1.0481±0.1100
Kidney	0.3096±0.0421	0.3161±0.0270	0.3230±0.0286	0.3045±0.0219
Thymus	0.0434±0.0042	0.0449±0.0046	0.0407±0.0044	0.0386±0.0051
Spleen	0.0581±0.0028	0.0597±0.0074	0.0585±0.0069	0.0562±0.0061
Adrenal gland	0.0033±0.0008	0.0031±0.0006	0.0034±0.0009	0.0031±0.0005
Testis	0.1637±0.0097	0.1674±0.0114	0.1611±0.0175	0.1446±0.0262*
Epididymis	0.0632±0.0053	0.0332±0.0036	0.0641±0.0029	0.0581±0.0057*
Seminal vesicle	0.2246±0.0385	0.2065±0.0309	0.2444±0.0161	0.2245±0.0148
prostate	0.0101±0.0015	0.0088±0.0018	0.0078±0.0019*	0.0085±0.0010

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 6. Absolute organ weights (g) of F_1 female mice born of F_0 male treated with diethanolamine on PND 70

Treatment (mg/kg)	control	20	80	320
Final Body Weight	21.37±1.53	21.79±1.87	19.63±1.62	20.29±2.64
Brain	0.4387±0.0257	0.4301±0.0134	0.4435±0.0266	0.4463±0.0179
Liver	1.1251±0.1599	1.2376±0.1525	1.1374±0.1365	1.2188±0.2901
Kidney	0.2859±0.0286	0.2835±0.0257	0.2721±0.0287	0.2760±0.0447
Thymus	0.0642±0.0046	0.0606±0.0081	0.0578±0.0068	0.0566±0.0105
Spleen	0.0730±0.0085	0.0756±0.0093	0.0716±0.0068	0.0679±0.0114
Adrenal gland	0.0054±0.0008	0.0059±0.0014	0.0060±0.0011	0.0061±0.0012
Uterus	0.0633±0.0145	0.0784±0.0401	0.0794±0.0217	0.0736±0.0301
Ovary	0.0065±0.0011	0.0068±0.0017	0.0071±0.0010	0.0065±0.0014

Mean±SD

Table 7. Physical development and reflex of F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)	control	20	80	320
Sensory hair	4.0±0	4.0±0	4.0±0	4.0±0
Pinna detachment	4.0±0	4.0±0	4.0±0	4.0±0
Visible pilation	7.0±0	7.0±0	7.4±1.21	9.1±6.61
Pinna reflex	7.0±0	7.0±0	7.0±0	7.0±0
Surface righting reflex	9.0±0	9.0±0	9.0±0	9.0±0
Negative geotaxis	9.0±0	9.0±0	9.2±0.60	9.0±0
Incisor eruption	10.0±0	10.0±0	10.0±0	10.0±0
Growth of claw	10.0±0	10.0±0	10.0±0	10.0±0
Midair righting reflex	14.0±0	14.0±0	14.0±0	14.0±0
Cliff avoidance	14.0±0	14.0±0	14.0±0	14.0±0
Wire grasping	14.2±0.63	14.0±0	14.1±0.30	14.0±0
Eye opening	14.0±0	14.0±0	14.0±0	14.0±0
Cornea reflex	20.0±0	20.0±0	20.0±0	20.0±0
Testis descent	21.5±4.14	21.3±0.50	23.1±1.30	24.2±2.71
Vaginal opening	30.0±0	30.0±0	31.8±3.92	30.1±0.30

Mean±SD

Table 8. Hot plate test of F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)		Control	20	80	320
Hot plate	male	22.22±2.514	31.39±7.208*	28.69±4.516*	28.88±4.100*
	female	16.18±4.605	20.37±7.364	26.97±4.451*	30.64±6.074*

Mean±SD (*Significantly different from the control, $p<0.05$)

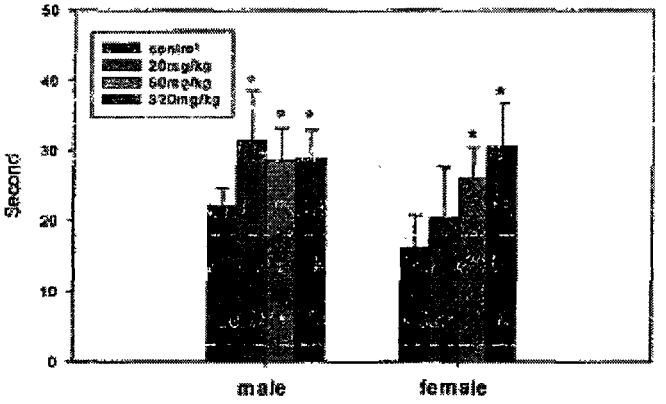

Figure 3. Hot plate test of F_1 mice born of F_0 male treated with diethanolamine. (*Significantly different from the control, $p<0.05$)

Table 9. Rota rod test of F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)	Control	20	80	320
male	1.05±0.517	0.68±0.474	1.16±0.620	1.13±0.625
female	1.45±0.584	1.03±0.521	0.99±0.447	1.03±0.416

Mean±SD

Table 10. Open field test of F_1 mice born of F_0 male treated with diethanolamine

Sex	Measurement	Control	20	80	320
Male	Total Distance(mm)	3480.5±888.2	3402.5±711.5	4027.2±779.5	3386.1±498.0
	V max	53.32±58.911	33.74±19.262	61.31±50.376	80.23±68.680
	V mean	6.16±0.999	5.68±1.171	6.93±1.415	6.40±1.793
Female	Total Distance(mm)	3560.2±331.3	3535.7±517.1	3722.9±477.8	3496.9±479.6
	V max	28.78±4.481	38.20±27.505	30.81±7.065	28.26±1.931
	V mean	5.93±0.524	6.29±0.962	6.54±0.847	6.06±0.806

Mean±SD

Table 11. Fear conditioning test of F_1 mice born of F_0 male treated with diethanolamine

Sex	Measurement	Treatment (mg/kg)			
		Control	20	80	320
Male	Context	55.11±21.526	40.64±14.962	43.54±14.896	46.67±23.942
	Cs	54.51±21.747	51.22±21.168	52.17±26.215	46.67±20.456
Female	Context	56.29±21.819	46.01±13.738	40.47±14.776	49.81±24.078
	Cs	34.07±5.340	34.99±2.994	34.30±11.525	34.06±4.595

Mean±SD

Table 12. Passive avoidance test of F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg)	Control	20	80	320
male	33.88±15.487	28.00±12.293	27.38±7.744	25.56±12.817
female	29.56±16.164	13.00±9.250	24.20±18.079	29.75±25.348

Mean±SD

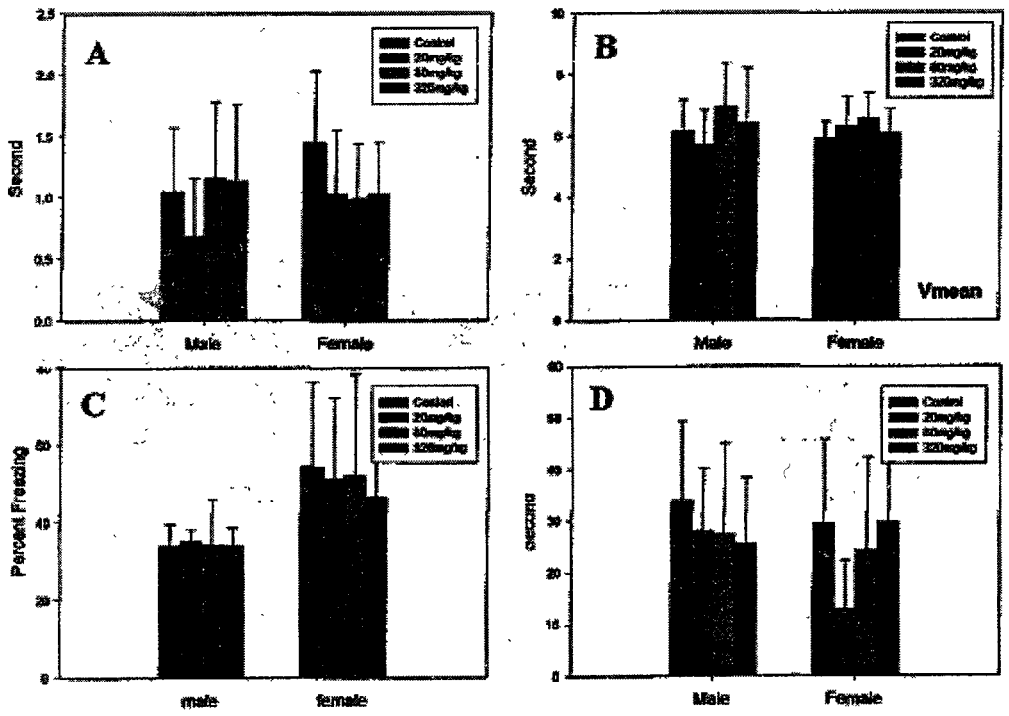

Figure 4. Rota-rod (A), open field (B), fear conditioning (C) and passive avoidance (D) test of F_1 mice born of F_0 male treated with diethanolamine

Table 13. Sperm motility and other parameter of sperm in F_0 male mice treated with diethanolamine

Treatment (mg/kg) / Parameter	control	20	80	320
Sperm motility (%)	76.90±7.88	63.00±9.85	62.40±9.14	59.80±11.27
Path velocity (㎛/s)	39.1±1.9	35.5±2.5	34.7±2.6	34.4±2.7
Prog. Velocity (㎛/s)	24.5±2.1	21.3±2.9	21.2±3.1	21.4±3.3
Track speed (㎛/s)	81.6±3.4	75.4±3.2	73.1±4.0	72.6±4.4
Lateral amplitude(㎛/s)	6.5±0.4	6.2±0.7	6.0±0.7	5.6±0.8
Beat frequency(Hz)	31.8±1.5	32.3±2.2	32.6±2.2	32.8±1.5
Straightness(%)	59.8±4.0	57.4±4.5	57.7±6.5	58.6±5.7
Linearity(%)	30.7±2.4	29.3±2.8	29.8±4.3	30.4±3.6
Elongation(%)	50.5±4.7	44.8±14.3	50.2±4.3	47.1±4.5

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 14. Sperm motility and other parameter of sperm in F_1 mice born of F_0 male treated with diethanolamine

Treatment (mg/kg) / Parameter	control	20	80	320
Sperm motility (%)	73.30±6.38	69.20±9.65	66.90±4.36	57.80±13.54
Path velocity (㎛/s)	39.2±3.1	36.0±1.8	34.6±2.3	33.8±2.9
Prog. Velocity (㎛/s)	23.4±2.7	19.2±6.7	21.2±2.8	20.1±4.0
Track speed (㎛/s)	82.7±4.9	77.2±3.3	75.2±3.6	72.6±4.2
Lateral amplitude(㎛/s)	6.7±0.5	6.7±0.6	6.3±0.6	5.8±0.7
Beat frequency(Hz)	33.1±1.7	33.6±1.3	33.9±1.2	34.0±1.8
Straightness(%)	55.3±4.4	56.0±3.8	58.1±5.5	56.1±7.2
Linearity(%)	28.2±2.7	28.1±1.9	29.0±3.0	28.5±4.8
Elongation(%)	46.3±1.8	45.3±2.3	45.1±3.2	47.3±5.5

Mean±SD (*Significantly different from the control, $p<0.05$)

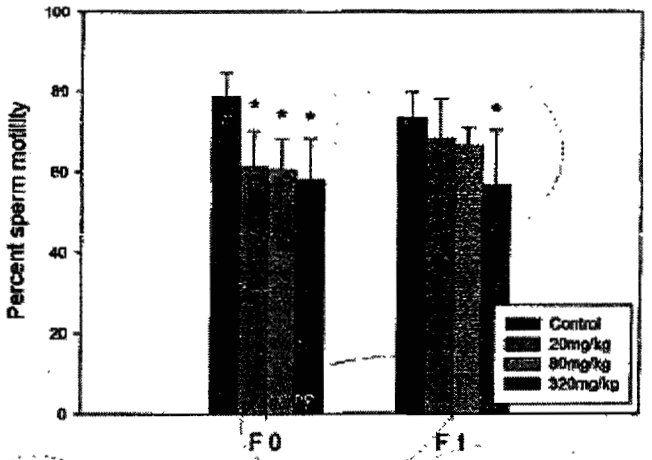

Figure 5. Sperm motility and other parameter of sperm in F_0 male mice treated with diethanolamine and F_1 mice born of F_0 male treated with diethanolamine. (*Significantly different from the control, $p<0.05$)

Table 15. Body weight of F_1 generation mice born of F_0 female treated with diethanolamine

Treatment (mg/kg)		Control	20	80	320
PND4	Male	2.84±0.19	2.75±0.45	2.92±0.30	2.45±0.39
	Female	2.90±0.35	2.72±0.50	2.80±0.42	2.47±0.31
PND7	Male	4.49±0.27	4.10±0.73	4.26±0.20	3.65±0.54*
	Female	4.66±0.50	3.88±0.53*	4.15±0.29	3.79±0.27*
PND14	Male	7.31±1.24	6.31±1.53	6.59±1.28	6.16±0.63
	Female	7.35±0.65	6.11±1.37*	6.34±0.63	6.19±0.32*
PND21	Male	10.16±0.92	9.66±1.38	9.48±0.77	8.21±1.08*
	Female	10.13±1.33	9.01±0.64	9.12±0.73	8.18±0.42*
PND28	Male	15.65±0.71	15.58±1.37	15.10±0.94	12.94±1.60*
	Female	13.54±0.52	13.04±0.34	13.64±1.17	12.06±0.54*
PND35	Male	18.68±1.10	19.14±1.68	18.93±0.90	17.44±1.13
	Female	16.73±0.80	15.89±0.80	16.29±1.15	15.41±0.40
PND42	Male	20.00±0.80	20.61±1.63	20.30±1.08	19.24±1.14
	Female	18.10±1.02	17.00±0.90	17.39±0.95	16.48±0.49*
PND49	Male	21.20±1.42	21.64±1.68	21.70±1.02	21.05±1.18
	Female	19.33±1.10	18.07±1.09	18.45±1.23	17.44±0.41*
PND56	Male	22.27±1.69	22.92±1.68	23.14±1.25	22.99±1.33
	Female	19.75±1.03	18.74±0.70	19.37±1.34	18.28±0.59
PND63	Male	23.40±1.71	24.21±1.85	24.53±1.25	23.95±1.25
	Female	20.48±1.14	19.27±0.69	20.10±1.61	18.89±0.38
PND70	Male	24.01±1.51	24.83±1.73	25.33±1.24	24.07±1.46
	Female	21.07±1.67	19.72±0.43	20.55±1.61	19.40±0.90

Mean±SD (*Significantly different from the control, $p<0.05$)

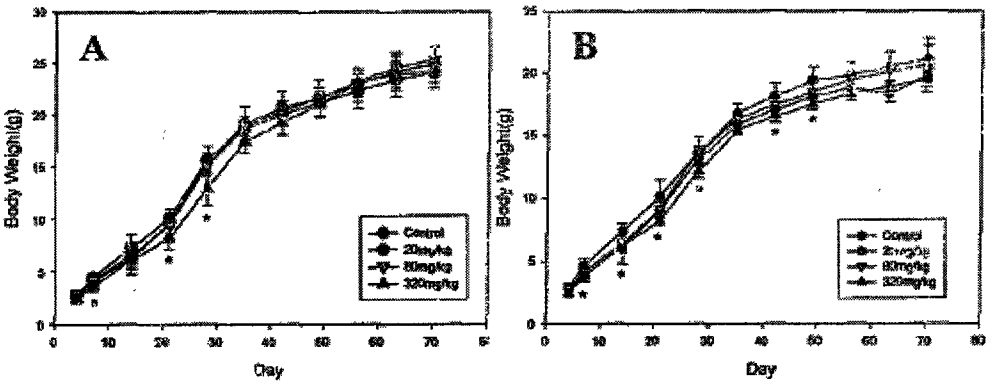

Figure 6. Change in body weight of F_1 male (A) and female (B) mice born of F_0 male treated with diethanolamine. (*Significantly different from the control, $p<0.05$)

Table 16. Absolute organ weights (g) of F_0 female mice treated with diethanolamine

Treatment (mg/kg)	control	20	80	320
Final Body Weight	25.03±0.71	25.32±1.00	24.94±0.96	26.79±0.92
Brain	0.3899±0.0735	0.4243±0.0386	0.4188±0.0295	0.4344±0.0173
Liver	1.9773±0.1204	2.0082±0.1499	2.2711±0.1194	2.8276±0.3114
Kidney	0.3364±0.0107	0.3409±0.0224	0.3586±0.0224	0.4113±0.0316
Thymus	0.0534±0.0077	0.0496±0.0114	0.0462±0.0091	[illegible]
Spleen	0.0695±0.0037	0.0693±0.0043	0.0755±0.0066	0.0972±0.0231
Adrenal gland	0.0058±0.0011	0.0060±0.0011	0.0069±0.0011	0.0069±0.0011
Uterus	0.0649±0.0112	0.0606±0.0165	0.0840±0.0416	0.0578±0.0225
Ovary	0.0097±0.0034	0.0086±0.0020	0.0099±0.0011	0.0096±0.0021

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 17. Absolute organ weights (g) of F_1 male mice born of F_0 female treated with diethanolamine on PND 21

Treatment (mg/kg)	control	20	80	320
Final Body Weight	9.05±1.03	9.99±1.34	9.27±0.60	8.45±0.82
Brain	0.3763±0.0285	0.3781±0.0142	0.3781±0.0215	0.3714±0.0317
Liver	0.4796±0.0815	0.5063±0.0929	0.4958±0.0877	0.4189±0.0765
Kidney	0.1319±0.0162	0.1473±0.0227	0.1453±0.0125	0.1320±0.0140
Thymus	0.0543±0.0151	0.0663±0.0143	0.0675±0.0079	0.0639±0.0080
Spleen	0.0651±0.0121	0.0803±0.0081	0.0574±0.0061	0.0684±0.0112
Adrenal gland	0.0026±0.0009	0.0026±0.0007	0.0022±0.0006	0.0021±0.0009
Testis	0.0448±0.0154	0.0415±0.0098	0.0428±0.0039	0.0380±0.0063
Epididymis	0.0075±0.0018	0.0095±0.0020	0.0090±0.0013	0.0081±0.0022
Seminal v. & prostate	0.0052±0.0019	0.0065±0.0022	0.0052±0.0007	0.0045±0.0011

Mean±SD

Table 18. Absolute organ weights (g) of F_1 female mice born of F_0 female treated with diethanolamine on PND 21

Treatment (mg/kg)	control	20	80	320
Final Body Weight	9.40±1.07	9.27±1.06	9.45±0.90	8.54±0.52
Brain	0.3667±0.0327	0.3677±0.0290	0.3790±0.0328	0.3772±0.0233
Liver	0.5348±0.0292	0.4362±0.0548	0.5401±0.0842	0.4584±0.0573
Kidney	0.1456±0.0085	0.1525±0.0202	0.1516±0.0118	0.1447±0.0076
Thymus	0.0612±0.0102	0.0646±0.0076	0.0643±0.0085	0.0606±0.0090
Spleen	0.0650±0.0155	0.0655±0.0097	0.0703±0.0099	0.0558±0.0075
Adrenal gland	0.0024±0.0005	0.0024±0.0006	0.0024±0.0006	0.0021±0.0005
Uterus	0.0090±0.0026	0.0081±0.0018	0.0088±0.0023	0.0067±0.0008
Ovary	0.0034±0.0010	0.0028±0.0006	0.0033±0.0009	0.0027±0.0009

Mean±SD

Table 19. Absolute organ weights (g) of F_1 male mice born of F_0 female treated with diethanolamine on PND 70

Treatment (mg/kg)	control	20	80	320
Final Body Weight	28.87±2.42	29.52±3.46	31.50±2.80	26.59±2.32
Brain	0.4427±0.0168	0.4181±0.0224	0.4277±0.0282	0.4373±0.0617
Liver	1.3242±0.1668	1.2807±0.1646	1.3231±0.1031	1.2405±0.1263
Kidney	0.3707±0.0351	0.3429±0.0381	0.3493±0.0326	0.3382±0.0192
Thymus	0.0410±0.0040	0.0403±0.0046	0.0426±0.0067	0.0365±0.0033
Spleen	0.0634±0.0105	0.0638±0.0078	0.0591±0.0074	0.0607±0.0087
Adrenal gland	0.0040±0.0009	0.0033±0.0005*	0.0028±0.0005*	0.0036±0.0011
Testis	0.1628±0.0077	0.1661±0.0161	0.1659±0.0089	0.1573±0.0180
Epididymis	0.0702±0.0056	0.0706±0.0042	0.0725±0.0035	0.0672±0.0058
Seminal vesicle	0.3237±0.0385	0.3166±0.0442	0.3076±0.0318	0.3053±0.0411
prostate	0.0111±0.0027	0.0096±0.0027	0.0112±0.0020	0.0095±0.0021

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 20. Absolute organ weights (g) of F_1 female mice born of F_0 female treated with diethanolamine on PND 70

Treatment (mg/kg)	control	20	80	320
Final Body Weight	21.21±1.42	20.34±0.59	21.45±1.41	20.29±1.21
Brain	0.4419±0.0215	0.4256±0.0298	0.4306±0.0239	0.4171±0.0899
Liver	1.0593±0.0805	1.0357±0.0604	1.0740±0.0982	0.9745±0.2171
Kidney	0.2674±0.0166	0.2596±0.0158	0.2702±0.0199	0.2667±0.0157
Thymus	0.0651±0.0028	0.0605±0.0046	0.0606±0.0061	0.0580±0.0081
Spleen	0.0660±0.0046	0.0682±0.0042	0.0729±0.0082	0.0687±0.0079
Adrenal gland	0.0065±0.0011	0.0056±0.0006	0.0059±0.0010	0.0062±0.0009
Uterus	0.1206±0.0505	0.1061±0.0493	0.0824±0.0278*	0.0700±0.0184*
Ovary	0.0074±0.0007	0.0069±0.0011	0.0076±0.0016	0.0073±0.0015

Mean±SD (*Significantly different from the control, $p<0.05$)

Table 21. Physical development and reflex of F_1 mice born of F_0 female treated with diethanolamine

Treatment (mg/kg)	control	20	80	320
Sensory hair	4.0±0	4.0±0	4.0±0	4.0±0
Pinna detachment	4.0±0	4.0±0	4.0±0	4.0±0
Visible pilation	7.0±0	7.0±0	7.0±0	7.0±0
Pinna reflex	7.0±0	7.0±0	7.0±0	7.0±0
Surface righting reflex	9.0±0	9.0±0	9.0±0	9.0±0
Negative geotaxis	9.0±0	9.0±0	9.0±0	9.0±0
Incisor eruption	10.0±0	10.0±0	10.0±0	10.0±0
Growth of claw	10.0±0	10.0±0	10.0±0	10.0±0
Midair righting reflex	14.0±0	14.0±0	14.0±0	14.0±0
Cliff avoidance	14.0±0	14.0±0	14.0±0	14.0±0
Wire grasping	14.0±0	14.0±0	14.0±0	14.0±0
Eye opening	14.0±0	14.0±0	14.0±0	14.0±0
Cornea reflex	20.0±0	20.0±0	20.0±0	20.0±0
Testis descent	21.4±1.34	22.8±1.64	23.4±0.88	24.7±3.64
Vaginal opening	30.8±0.45	30.8±0.45	31.4±1.88	31.9±0.90

Mean±SD

Table 22. Hot plate test of F_1 mice born of F_0 female treated with diethanolamine

Treatment (mg/kg)		Control	20	80	320
Hot plate	male	19.84±6.195	33.35±19.253	25.03±3.151	29.89±5.743
	female	18.29±3.185	24.89±6.287	25.79±6.289	28.99±9.704

Mean±SD

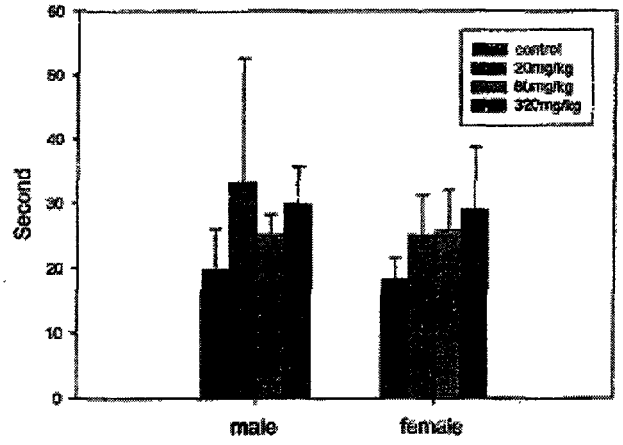

Figure 7. Hot plate test of F_1 mice born of F_0 female treated with diethanolamine

Table 24. Open field test of F_1 mice born of F_0 female treated with diethanolamine

Sex	Measurement	Control	20	80	320
Male	Total Distance(mm)	2890.4±496.4	4043.2±1810.2	3232.8±1024.6	3148.3±1231.6
	V max	28.38±5.575	50.93±29.442*	29.49±4.711	26.13±4.974
	V mean	4.81±0.827	8.21±3.478	6.51±3.705	5.89±2.810
Female	Total Distance(mm)	3357.7±596.4	2917.3±1066.6	3217.5±847.3	[illegible].73.3±627.2
	V max	27.32±1.943	29.17±6.200	29.25±3.202	31.65±3.889
	V mean	5.58±1.006	5.44±1.246	5.63±0.979	6.95±1.043

Mean±SD

Table 25. Fear conditioning test of F_1 mice born of F_0 female treated with diethanolamine

Sex	Measurement	Control	20	80	320
Male	Context	34.73±5.827	36.10±9.713	33.67±3.280	35.56±9.737
	Cs	39.02±14.507	29.55±5.251	29.79±5.869	29.98±5.023
Female	Context	69.43±11.549	60.67±19.662	63.88±18.015	65.70±17.700
	Cs	35.17±12.090	32.58±4.216	31.59±3.836	32.50±3.964

Mean±SD

Table 26. Passive avoidance test of F_1 mice born of F_0 female treated with diethanolamine

Treatment (mg/kg)	Control	20	80	320
male	35.75±19.293	26.63±11.747	22.50±10.515	27.44±11.717
female	54.71±69.980	28.71±18.590	27.22±14.646	37.25±21.486

Mean±SD

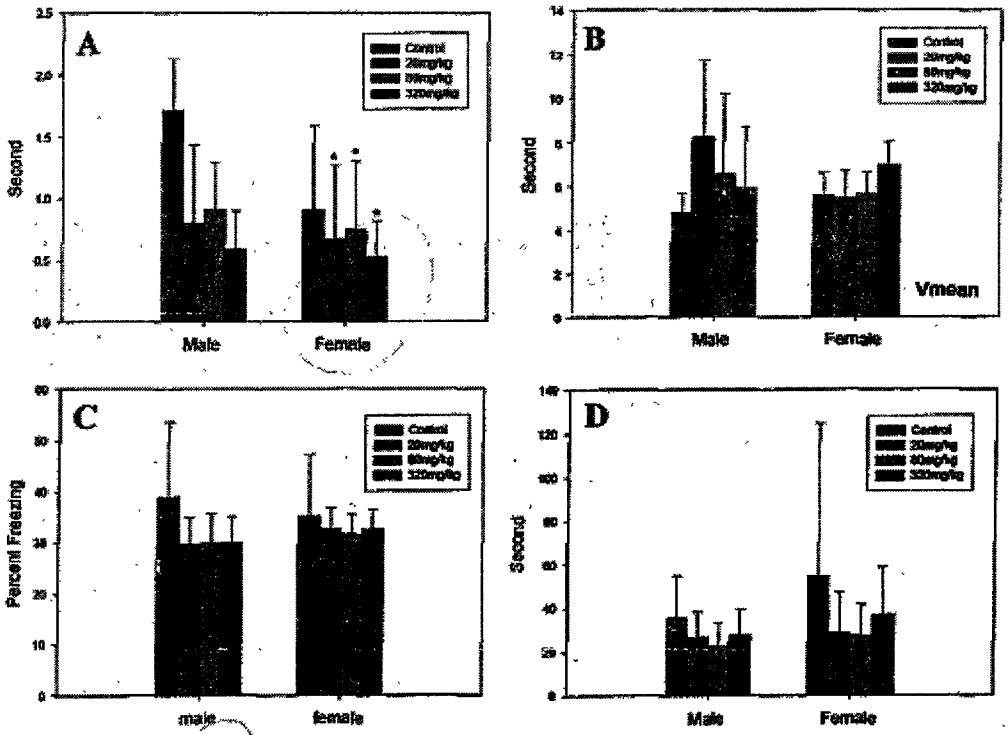

Figure 8. Rota-rod (A), open field (B), fear conditioning (C) and passive avoidance (D) test of F_1 mice born of F_0 female treated with diethanolamine

Table 27. Sperm motility and other parameter of sperm in F_1 mice born of F_0 female treated with diethanolamine

Parameter \ Treatment (mg/kg)	control	20	80	320
Sperm motility (%)	70.60±11.81	65.50±7.89	64.60±7.14	67.80±8.97
Path velocity (㎛/s)	37.8±4.3	35.0±1.9	37.3±1.7	36.8±2.0
Prog. Velocity (㎛/s)	23.5±3.6	21.3±2.3	23.4±2.4	21.7±1.8
Track speed (㎛/s)	78.9±7.0	75.1±2.9	77.4±2.5	76.4±4.2
Lateral amplitude(㎛/s)	6.1±0.8	6.1±0.5	6.4±0.7	6.5±0.8
Beat frequency(Hz)	32.0±1.6	33.2±1.1	31.6±2.0	32.2±1.2
Straightness(%)	56.9±8.7	58.1±4.2	59.8±4.3	55.2±4.4
Linearity(%)	30.4±2.4	29.6±2.5	31.0±2.7	28.8±2.3
Elongation(%)	49.8±3.2	44.8±2.2	45.6±2.3	46.7±4.0

Mean±SD

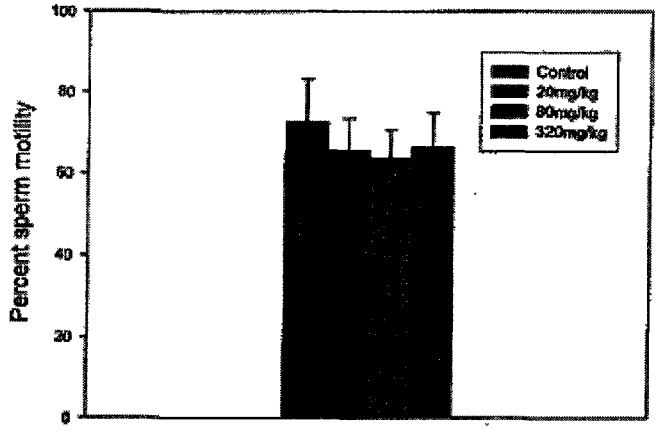

Figure 9. Sperm motility and other parameter of sperm in F_1 mice born of F_0 female treated with diethanolamine