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Female rats are less susceptible during puberty to the lethal effects of percutaneous exposure to VX

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ABSTRACT

Nerve agents with low volatility such as VX are primarily absorbed through the skin when released during combat or a terrorist attack. The barrier function of the stratum corneum may be compromised during certain stages of development, allowing VX to more easily penetrate through the skin. However, age-related differences in the lethal potency of VX have yet to be evaluated using the percutaneous (pc) route of exposure. Thus, we estimated the 24 and 48 h median lethal dose for pc exposure to VX in male and female rats during puberty and early adulthood. Pubescent, female rats were less susceptible than both their male and adult counterparts to the lethal effects associated with pc exposure to VX possibly because of hormonal changes during that stage of development. This study emphasizes the need to control for both age and sex when evaluating the toxicological effects associated with nerve agent exposure in the rat model.

1. Introduction

Aum Shinrikyo, the religious cult responsible for the release of the nerve agent sarin in the Tokyo subway system, used VX in as many as five assassination attempts in the mid-1990s [29]. Morimoto et al. [18] describe the symptoms (miosis, excessive perspiration, diarrhea, hypothermia, muscle fasciculations and pulmonary edema) experienced by one of the victims before he died sixteen days after being injected in the neck with VX. Both nerve agents cause lethality by irreversibly binding to acetylcholinesterase (AChE) and inhibiting the hydrolysis of the neurotransmitter acetylcholine; however, VX is three orders of magnitude less volatile than sarin and primarily enters the circulatory system through the skin rather than the lungs when released in a battlefield or terrorist situation (reviewed in Ref. [19])

The outermost layer of the skin, or stratum corneum, is the major barrier against percutaneous (pc) exposure to VX, and variables related to its function (hydration, lipid content, pH, thickness, sebum production and transepidermal water loss [TEWL]) change with age [3,14,15]. As reviewed in Ref. [28], the barrier function of the stratum corneum is not fully competent at time of birth. Infants (defined as individuals between birth and 3 years of age) have thinner, less acidic and more hydrated strata cornea than young adults (defined as individuals between 20 and 40 years of age) with similar or higher TEWL values depending on the anatomic location, suggesting that VX may be able to more easily penetrate through the skin during the first years of life. In fact, Ngawhirunpat et al. [21] showed that the permeability coefficients for lipophilic compounds ($\log K_{ow} = 2.09$ for VX; [20]) through intact skin are higher for postnatal day (PND) 5 rats than for any other age group (up to PND 180).

The barrier function of the stratum corneum may also be compromised during puberty. Compared to adults, pubescent children (defined as individuals between 10 and 14 years of age) have drier skin with similar or higher TEWL values depending on the anatomic location [1]. In addition, the surge of hormones during puberty

Abbreviations: (AChE), acetylcholinesterase; (ANOVA), analysis of variance; (BARDA), Biomedical Advanced Research and Development Authority; (CI), confidence interval; (LD_{50}), median lethal dose; (pc), percutaneous or percutaneously; (PND), postnatal day; (SC), subcutaneous or subcutaneously; (TEWL), transepidermal water loss; (USAMRICD), US Army Medical Research Institute of Chemical Defense.

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triggers a rapid increase in sebum production and causes females, but not males, to accumulate a thick layer of subcutaneous (sc) fat (reviewed in Ref. [13]). Thus, pubescent children are likely to be more vulnerable than adults to the toxic effects associated with pc exposure to VX.

The goal of this study was to evaluate the lethal potency of VX during puberty and early adulthood in the rat model. Using a stagewise, adaptive dose design, we estimated the 24 and 48 h LD_{50} for pc exposure to VX in male and female rats for two different age groups (PND 42 and 70). PND 70 rats were chosen to model the typical combat soldier between 18- and 25-years old. Unfortunately, we were unable to estimate the LD_{50} for younger age groups of rats as the digital syringe was not sensitive enough to apply the picoliter quantities of VX that would be needed due to the low body weight of rats younger than PND 42. Nevertheless, we found pubescent, female rats to be less susceptible (higher LD_{50} values) than their adult counterparts to the lethal effects associated with pc exposure to VX. However, age-related differences in the lethal potency of VX were not observed with the male rats.

2. Methods

2.1. Animals

Male and female Sprague-Dawley rats (CD IGS) were purchased from Charles River Laboratories International, Inc. (Kingston, NY) and divided into two groups based on their age (PND 42 and 70). The pre-exposure weights of each age group and sex are listed in Table 1. Rats were individually housed in temperature- and humidity-controlled rooms ($21 \pm 2\,^{\circ}C$ and $60 \pm 20\%$, respectively) under a 12:12 h light:dark cycle (lights on at 06:00). Food and water were available ad libitum. The experimental protocol was approved by the Animal Care and Use Committee at the US Army Medical Research Institute of Chemical Defense (USAMRICD), and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, the most current Public Health Safety Policy on Humane Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

2.2. Animal preparation

Approximately 24 h prior to the exposures, the fur on the right flank of each rat was clipped with a Dander-free Clipper System (Hazard Technology; Pasadena, MD) equipped with an Oster A5 clipper and a #40 CryogenX blade (Boca Raton, FL). Care was taken to limit razor burn, and VX was not applied to an area with visible abrasions. On the morning of the exposures, the rats were moved to a procedure room with a bank of chemical fume hoods. A circle (2.5 cm in diameter) was drawn on the clipped flank of each rat with a black permanent marker (Sharpie; Downers Grove, IL). Each rat was then fitted with an Elizabethan collar (Lomir Biomedical, Inc.; Malone, NY), placed in a polycarbonate cage (16.5 cm wide × 19 cm long × 21.5 cm high) lined with an iso-PAD (Harland Laboratories, Inc.; Indianapolis, IN) and moved into one of the hoods where it remained for the rest of the study.

2.3. Agent application

VX (ethyl-*S*-dimethylaminoethyl methylphosphonothiolate) was obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD). Using methods similar to those described by Clarkson et al. [4], VX was applied in its neat (undiluted) form using a digital 0.5 μl syringe with 2.5 nl increments (Hamilton Laboratory Products; Reno, NV) to the center of the circle drawn on each un-anesthetized rat. The volume of VX applied to each rat ranged from 2.5 to 115 nl, and the specific gravity of VX (1.01 g/ml) along with each rat's body weight was used to calculate the dose that was administered. Exposures were conducted between 09:00 and 11:00, and toxic signs (abnormal mouth movements, convulsions, forelimb clonus, lacrimation, muscle fasciculations, salivation and tremors) were continuously monitored throughout the business day. Survivors were euthanized at 48 h post-exposure with Fatal-Plus (Vortech Pharmaceuticals, Ltd.; Dearborn, MI), and tissue samples were collected and archived in a $-80\,^{\circ}C$ freezer for future analyses.

2.4. LD_{50} estimations

The 24 and 48 h lethality for pc exposure to VX was estimated by establishing dose-response curves, and each curve was generated using a stagewise, adaptive dose design [8–10]. In the first stage, three doses ($n = 2$–3 rats/dose) were selected to span the predicted range of lethality from 0 to 100%. The lethality results from the first stage were used to select doses ($n = 1$–3 rats/dose) for the second stage. In the subsequent stages, doses were selected to further focus on a 50% lethality response and/or to better estimate the dose–response curve. After each stage, probit dose–response models using maximum likelihood methods were fitted to the combined data for all stages [9,11]. The stage process continued until the half width of the 95% confidence interval (CI) defined as (Upper Bound–Lower Bound)/($2 \times LD_{50}$) for the 48 h LD_{50} was less than 0.4 or a maximum of 30 rats was used for each age group and sex.

2.5. Statistical analyses

The 24 and 48 h LD_{50} values for pc exposure to VX were estimated by probit analysis using SAS NLIN (SAS Institute, Cary, NC) and the specialized programs of Feder et al. [8–10]. The delta method was used to compute a 95% CI for each LD_{50} value. Comparisons between male and female LD_{50} values for each age group, as well as comparisons between age groups within each sex, were made by calculating a comparative ratio of the two LD_{50} values along with a 95% CI for that ratio. This approach is a variation of the two-sided *Z*-test ($\alpha = 0.05$). If the 95% CI for the comparative ratio did not encompass the value of 1.0, then the LD_{50} values of the age groups or sexes being compared were determined to be significantly ($p < 0.05$) different. SigmaPlot 12.3 (Systat Software, San Jose, CA) was used to plot the dose–response curves.

3. Results

3.1. LD_{50} estimates

Table 2 shows the 24 and 48 h LD_{50} values for each group of rats pc exposed to VX. For PND 42 rats, males had significantly lower LD_{50} values than females. No sex differences were observed for PND 70 rats. For males, no age-related differences were observed. For females, however, the 48 h LD_{50} value for PND 70 rats was lower than the value for PND 42 rats. No other significant differences were observed for females.

3.2. Dose–response curves

Fig. 1 shows the dose–response curves for each group of rats pc exposed to VX. No differences were observed for males. For females, however, the dose–response curves for PND 42 rats were shifted to the right compared to PND 70 rats, implying that VX was less potent when administered to rats during puberty.

Table 1
Body weight (mean ± standard deviation) for male and female rats during puberty and early adulthood. The body surface area for each rat was calculated using the equation of Spiers and Candas [27], which takes into account the different stages of development. This value was then divided by the rat's body weight to give a body surface area to body weight ratio. A two-way analysis of variance (ANOVA) with age group and sex as factors was conducted to determine differences in these ratios. There was a significant interaction between the two factors ($F(1,113) = 14.6$, $p < 0.001$); thus, a t-test was conducted for each sex to determine differences between age groups.

	Male				Female			
Group	Weight (g)	Surface area (cm^2)	Ratio (cm^2/g)	*N*	Weight (g)	Surface area (cm^2)	Ratio (cm^2/g)	*N*
PND 42	231 ± 14	380 ± 17	1.63 ± 0.03*	26	170 ± 7	302 ± 10	1.77 ± 0.02*	24
PND 70	353 ± 13	516 ± 14	1.46 ± 0.01	23	232 ± 12	379 ± 15	1.63 ± 0.02	24

* Significant ($p < 0.05$) differences.

Table 2
24 and 48 h LD_{50} values and 95% CI for male and female rats pc exposed to VX during puberty or early adulthood. Comparisons were made between sexes for each time and age group, and significant ($p < 0.05$) differences are highlighted in bold.

	Group	24 h		48 h	
		Male	Female	Male	Female
LD_{50} (μg/kg)95% CISlope ± SE*N*	PND 42	**41.2** 32.9–51.7 8.8 ± 3.9 26	**69.9** 61.1–80.0 14.5 ± 3.6 24	**41.2** 32.9–51.7 8.8 ± 3.9 26	**69.9*** 61.1–80.0 14.5 ± 3.6 24
LD_{50} (μg/kg)95% CISlope ± SE*N*	PND 70	43.1 38.5–48.2 19.2 ± 5.8 23	50.8 36.3–71.2 4.5 ± 3.8 24	38.4 33.5–44.0 16.0 ± 2.8 23	42.8 29.3–62.6 4.6 ± 2.8 24

* Significant ($p < 0.05$) differences between age groups for each time and sex.

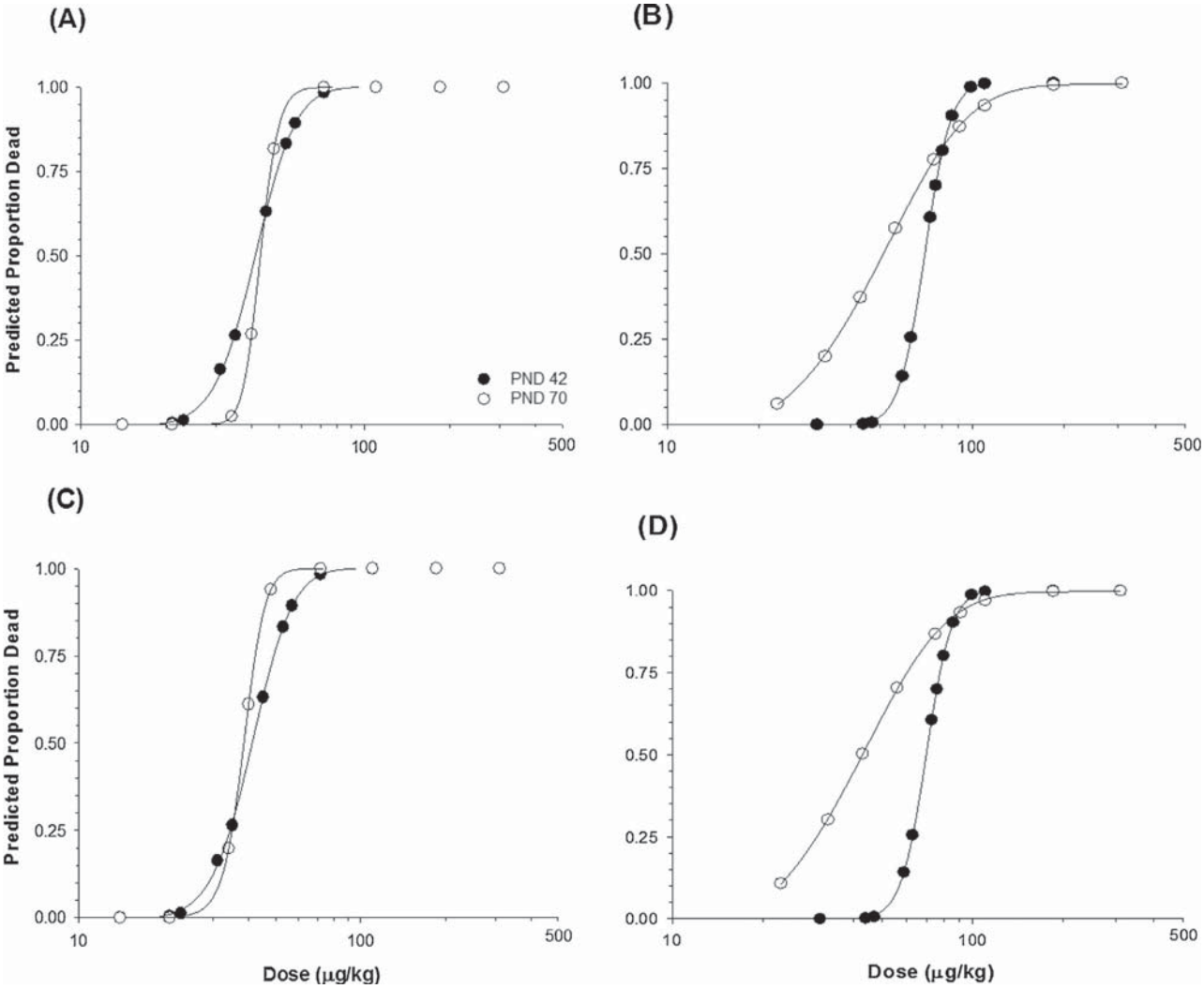

Fig. 1. Dose–response curves for male and female rats pc exposed to VX during puberty or early adulthood. (A) 24 h—males; (B) 24 h—females; (C) 48 h—males; (D) 48 h—females.

3.3. Toxic signs

The first toxic sign observed for nearly 80% of the rats was abnormal mouth movements (chewing, oral tonus and/or tongue fasciculations). Approximately 22% of the rats that died from pc exposure to VX had generalized motor convulsions as defined by Racine [22], and no statistical differences (Fisher's exact test;

$p > 0.05$) were observed between groups in terms of the number of rats that experienced convulsions. For those that died during the first business day, the latency to death was 85 ± 54 min (range: 12–221 min) for PND 42 rats and 136 ± 103 min (range: 15–372 min) for PND 70 rats. There were no deaths among the pubescent age group between 24 and 48 h post-exposure.

4. Discussion

Skin is the largest and outermost organ of the body comprising over 15% of an animal's body mass [7]. Its major functions are to protect against water loss and prevent exogenous substances like VX from entering the body. These functions, however, may be compromised during an animal's development including puberty. Thus, we estimated LD_{50} values for pc exposure to VX in male and female rats at two different times in development (PND 42 and 70). We found that the lethal potency of VX increased with the age of female, but not male, rats.

The 24 h LD_{50} values for pc exposure to VX were approximately twice the values that we estimated for each age group and sex of rats using the sc route of exposure and VX diluted in normal saline (PND 42 (males and females): 25.3 and 24.5 μg/kg, respectively; PND 70 (males and females): 19.0 and 21.1 μg/kg, respectively; [31]) demonstrating the level of protection that is provided by the skin. Misik et al. [17] found VX to be seven times more lethal in male rats (6–7 weeks old based on the reported body weight range) when administered sc rather than pc. However, they used a different strain of rats and needed to dilute VX in 0.01% hexane to have a large enough volume to pipette it onto the skin. The ability to administer VX in its neat form was essential for us as some solvents reduce its dermal absorption depending upon their chemical properties [5].

Pubescent, female rats were less susceptible than both their adult and male counterparts to the lethal effects associated with pc exposure to VX. Estradiol becomes detectable in female rats on PND 28 and reaches adult levels by PND 48 [30]. The surge of this estrogen hormone during puberty may have, contrary to our hypothesis, improved the barrier function of the stratum corneum in female rats. Harvell et al. [12] reported that TEWL values were lower on the back and forearms of women just prior to ovulation when estrogen levels are at their highest. In addition, estrogen replacement therapy minimized the visible signs of aging in postmenopausal women by maintaining the thickness and moisture content of the skin (reviewed in Ref. [24]).

The surge of estrogen during puberty may have also had a protective effect irrespective of the skin as demonstrated by Smith et al. [25], who showed that the 24 h LD_{50} value for sc exposure to sarin was highest in female rats during the proestrus stage of their hormonal cycle. Moreover, pretreating male rats with ethylestrenol caused a 150% increase in the LD_{50} value for sc exposure to parathion [23], an organophosphorus pesticide that inhibits AChE similar to VX but with less potency. Pretreatment with diethylstilbestrol also increased the efficacy of HI-6, an oxime that reactivates AChE, in castrated, male rats sc exposed to the nerve agents soman or tabun [16]. Given that estrogen therapy has been shown to be beneficial for a number of neurological disorders (reviewed in Ref. [26]), additional research is warranted to elucidate the mechanism(s) by which estrogen might afford protection against exposure to VX in the rat model.

As reviewed in Ref. [6], sex differences in the barrier function of the stratum corneum are mainly limited to infants. Thus, it seems unsurprising that there were no statistical differences between the LD_{50} values for male and female rats during early adulthood. Adult, male and female rats were also equally susceptible to the lethal effects associated with sc [31] or whole-body [2] exposure to VX.

In conclusion, age-related differences in susceptibility to the lethal effects associated with pc exposure to VX were observed with female, but not male, rats. The surge of estrogen hormones during puberty may afford female rats an innate protection against nerve agent exposure; however, this needs to be followed up with additional research. Nevertheless, this study underscores the need to carefully control for both the age and sex of the animal when evaluating the toxicological effects associated with nerve agent exposure.

Declaration of interest

BARDA had no involvement in the study design nor in the collection, analysis and interpretation of data or the decision to write this manuscript and submit it for publication. The views expressed in this manuscript are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense or the US Government.

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References

[1] N. Akutsu, M. Ooguri, T. Onodera, Y. Kobayashi, M. Katsuyama, N. Kunizawa, T. Hirao, J. Hosoi, Y. Masuda, S. Yoshida, M. Takahashi, T. Tsuchiya, H. Tagami, Functional characteristics of the skin surface of children approaching puberty: age and seasonal influences, Acta Derm. Venereol. 89 (2009) 21–27.
[2] B.J. Benton, J.M. McGuire, D.R. Sommerville, P.A. Dabisch, E.M. Jakubowksi, K.L. Matson, R.J. Mioduszewski, S.A. Thomson, C.L. Crouse, Effects of whole-body VX vapor exposure on lethality in rats, Inhal. Toxicol. 18 (2006) 1091–1099.
[3] E. Boireau-Adamezyk, A. Baillet-Guffroy, G.N. Stamatas, Age-dependent changes in stratum corneum barrier function, Skin Res. Technol. 20 (2014) 409–415.
[4] E.D. Clarkson, S.M. Schulz, R.F. Railer, K.H. Smith, Median lethal dose determination for percutaneous exposure to soman and VX in guinea pigs and the effectiveness of decontamination with M291 SDK or SANDIA foam, Toxicol. Lett. 212 (2012) 282–287.
[5] C.H. Dalton, I.J. Hattersley, S.J. Rutter, R.P. Chilcott, Absorption of the nerve agent VX (*O*-ethyl-*S*-[2(di-isopropylamino) ethyl] methyl phosphonthioate) through pig, human and guinea pig skin in vitro, Toxicol. In Vitro 20 (2006) 1532–1536.
[6] R. Darlenski, J.W. Fluhr, Influence of skin type, race, sex, and anatomical location on epidermal barrier function, Clin. Dermatol. 30 (2012) 269–273.
[7] M.D. Delp, M.V. Evans, C. Duan, Effects of aging on cardiac output, regional blood flow, and body composition in Fischer-344 rats, J. Appl. Physiol. 85 (1998) 1813–1822.
[8] P.I. Feder, D.W. Hobson, C.T. Olson, R.L. Joiner, M.C. Matthews, Stagewise, adaptive dose allocation for quantal response dose–response studies, Neurosci. Biobehav. Rev. 15 (1991) 109–114.
[9] P.I. Feder, C.T. Olson, D.W. Hobson, M.C. Matthews, R.L. Joiner, Stagewise, group sequential experimental designs for quantal responses. One-sample and two-sample comparisons, Neurosci. Biobehav. Rev. 15 (1991) 129–133.
[10] P.I. Feder, C.T. Olson, D.W. Hobson, M.C. Matthews, R.L. Joiner, Statistical analysis of dose–response experiments by maximum likelihood analysis and iteratively reweighted nonlinear least squares regression techniques, Drug Inf. J. 25 (1991) 323–334.
[11] D.J. Finney, Statistical aspects of monitoring for dangers in drug therapy, Methods Inf. Med. 10 (1971) 1–8.

[12] J. Harvell, I. Hussona-Saeed, H.I. Maibach, Changes in transepidermal water loss and cutaneous blood flow during the menstrual cycle, Contact Dermatitis 27 (1992) 294–301.
[13] A. Leung, S. Balaji, S.G. Keswani, Biology and function of fetal and pediatric skin, Facial Plast Surg. Clin. North Am. 21 (2013) 1–6.
[14] S. Luebberding, N. Krueger, M. Kerscher, Age-related changes in skin barrier function–quantitative evaluation of 150 female subjects, Int. J. Cosmet. Sci. 35 (2013) 183–190.
[15] S. Luebberding, N. Krueger, M. Kerscher, Age-related changes in male skin: quantitative evaluation of one hundred and fifty male subjects, Skin Pharmacol. Physiol. 27 (2014) 9–17.
[16] P.M. Lundy, J.C. Goulet, B.T. Hand, Hormone- and dose schedule-dependent protection by HI-6 against soman and tabun poisoning, Fundam Appl. Toxicol. 12 (1989) 595–603.
[17] J. Misik, R. Pavlikova, J. Cabal, K. Kuca, Acute toxicity of some nerve agents and pesticides in rats, Drug Chem. Toxicol. 38 (2015) 32–36.
[18] F. Morimoto, T. Shimazu, T. Yoshioka, Intoxication of VX in humans, Am. J. Emerg. Med. 17 (1999) 493–494.
[19] N. Munro, K.R. Ambrose, A.P. Watson, Toxicity of the organophosphate chemical warfare agents GA, GB, and VX: implications for public protection, Environ. Health Perspect. 102 (1994) 18–37.
[20] N.B. Munro, S.S. Talmage, G.D. Griffin, L.C. Waters, A.P. Watson, J.F. King, V. Hauschild, The sources, fate, and toxicity of chemical warfare agent degradation products, Environ. Health Perspect. 107 (1999) 933–974.
[21] T. Ngawhirunpat, H. Yoshikawa, T. Hatanaka, T. Koizumi, I. Adachi, Age-related changes in skin permeability of hydrophilic and lipophilic compounds in rats, Pharmazie 56 (2001) 231–234.
[22] R.J. Racine, Modification of seizure activity by electrical stimulation: II. Motor seizure, Electroencephalogr. Clin. Neurophysiol. 32 (1972) 281–294.
[23] C.P. Robinson, P.W. Smith, C.R. Crane, J.K. McConnell, L.V. Allen, B.R. Endecott, The protective effects of ethylestrenol against acute poisoning by organophosphorus cholinesterase inhibitors in rats, Arch. Int. Pharmacodyn. Ther. 231 (1978) 168–176.
[24] M.G. Shah, H.I. Maibach, Estrogen and skin: an overview, Am. J. Clin. Dermatol. 2 (2001) 143–150.
[25] C.D. Smith, L.K. Wright, G.E. Garcia, R.B. Lee, L.A. Lumley, Hormone-dependence of sarin lethality in rats: sex differences and stage of the estrous cycle, Toxicol. Appl. Pharmacol. 287 (2015) 253–257.
[26] R.D. Spence, R.R. Voskuhl, Neuroprotective effects of estrogens and androgens in CNS inflammation and neurodegeneration, Front. Neuroendocrinol. 33 (2012) 105–115.
[27] D.E. Spiers, V. Candas, Relationship of skin surface area to body mass in the immature rat: a reexaminiation, J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 56 (1984) 240–243.
[28] G.N. Stamatas, J. Nikolovski, M.C. Mack, N. Kollias, Infant skin physiology and development during the first years of life: a review of recent findings based on in vivo studies, Int. J. Cosmet. Sci. 33 (2011) 17–24.
[29] A.T. Tu, Aum Shinrikyo's chemical and biological weapons: more than sarin, Forensic Sci. Rev. 26 (2014) 115–120.
[30] C.S. Vetter-O'Hagen, L.P. Spear, Hormonal and physical markers of puberty and their relationship to adolescent-typical novelty-directed behavior, Dev. Psychobiol. 54 (2012) 523–535.
[31] L.K. Wright, R.B. Lee, N.M. Vincelli, C.E. Whalley, L.A. Lumley, Comparison of the lethal effects of chemical warfare nerve agents across multiple ages, Toxicol. Lett. 241 (2016) 167–174.