Acta Scientiae Veterinariae
ISSN: 1678-0345
ActaSciVet@ufrgs.br
Universidade Federal do Rio Grande do Sul
Brasil

Ali Shahbazfar, Amir; Mohammadpour, Hemn; Ahangaran Rajabi, Nastaran; Asmand, Mohsen
Comparative Pathologic and Stereologic Study of Respiratory System in Mice Following Administration of 3-Methyl Indole Dissolved in Propylene Glycol and Cremophor EL and LD50 Determination
Acta Scientiae Veterinariae, vol. 39, núm. 4, 2011, pp. 1-8
Universidade Federal do Rio Grande do Sul
Porto Alegre, Brasil

Acta Scientiae Veterinariae, 2011. 39(4): 993.

ORIGINAL ARTICLE
Pub. 993

ISSN 1679-9216 (Online)

# Comparative Pathologic and Stereologic Study of Respiratory System in Mice Following Administration of 3-Methyl Indole Dissolved in Propylene Glycol and Cremophor EL and LD50 Determination

**Amir Ali Shahbazfar[1], Hemn Mohammadpour[1], Nastaran Ahangaran Rajabi[2] & Mohsen Asmand[1]**

**ABSTRACT**

***Background*:** 3-Methyl indole(Skatole, 3-MI) is produced by microfloral fermentation of tryptophan in rumen and production of that has relation with the onset of respiratory problems in cattle. The aim of this study was to compare complications of 3-MI dissolved in two solvents, Cremophor and Propylene Glycol on BALB/c mice by histopathologic and stereologic studies.

***Materials, Methods & Results*:** Female Balb/C mice 56 days of age (23 - 27 g) were divided into 13 groups of eight and had access to food and water ad libitum. Mice were housed in plastic cages (4 per cage) with wire bar lids on bedding of sawdust in an air-conditioned room with 12 h light: dark cycles. After a 7-day acclimation period, for 5 groups, Mice were injected with 400, 500, 600, 700 and 800 mg/kg from solution of 3-MI in propylene glycol and similarly for 5 other groups with Cremophor. As well as 2 groups were injected with Propylene glycol and Cremophor with the dosage that is necessary for solving the highest dose of 3-MI. One group received normal saline with the volume equal with Cremophor (drug vehicle) group. Briefly, 125 mg of 3-MI was dissolved per mL of sterile filtered Propylene glycol and 30 mg of 3-MI was dissolved per mL of sterile filtered Cremophor. Once injected intra peritoneally, mice were monitored 3-4 h for external reaction signs from the injection. LD50 was determined 610.16 mg/kg 3-Methylindole and 687.5 mg/kg 3-Methylindole dissolved in Propylene glycol and Cremophor respectively with linear regression. Increase in respiration rate, tachypnea and shallow respiration was observed in clinical examination. In necropsy, alveolar and bronchial hyperemia was significant in lungs. Some superficial wounds were observed in upper respiratory airways. Trachea and Hungarian airways were severely hyperemic. There were some debris's in airways canals. We couldn't find any lesion in other organs. Histopathology showed noticeable alveolar edema and emphysema, type I pneumocytes necrosis, Slight endothelial lesions, rare degeneration of type II pneumocytes and raised nutrophil infiltration around vessels were observed in higher dosages of both groups. Moderate interstitial edema, hyperemia and increase of alveolar macrophages were observed too. Increase of lymphocytes was noticed after the dose 600 mg/kg moderately on a dose dependent manner. There was a Hyaline membrane in the wall of air sacs. Endothelial capillary damage in Cremophor dissolved 3-MI groups were slightly lower. Although damage to the vessels was not that much progressed in both groups. Stereologic study of type II pneumocytes showed a significant increase in the percentage of them in alveoli of treatment groups in a dose dependent manner compare to control and drug vehicle groups. At the highest dose (800 mg/kg) the group with Propylene glycol as solvent had significant difference with Cremophor dissolved 3-Methyl indole group and epithelialization was more severe in that. Bronchiole injuries raised in a dose dependent manner in both treatment groups.

***Discussion*:** The results of this research indicate that intraperitoneal infusion of 3-MI dissolved in Propylene glycol and Cremophor cause noticeable changes in the respiratory systems of mice. Effects of 3-MI in Propylene glycol is more severe than 3-MI dissolved in Cremophor but type of the lesions is not different. Bronchiolar lesions were moderate compare to the effects of 3-MI in some other animals. Severity of vascular lesions in Propylene glycol dissolved 3-MI was greater than groups that received the Cremophor dissolved 3-MI. Although the vascular injuries were not that much noticeable at all.

**Keywords:** 3-Methylindole, Balb/c mice, pulmonary edema, emphysema, LD50.

Received: March 2011 www.ufrgs.br/actavet Accepted: July 2011

[1]Department of Pathobiology, Faculty of Veterinary Medicine,University of Tabriz,Tabriz, Iran. [2]Department of Animal breeding, Faculty of Agricultural Science, University of Tabriz, Tabriz, Iran. CORRESPONDENCE: A.A. Shahbazfar [Shahbazfar@tabrizu.ac.ir - Phone: +98 (411) 3392339]. Department of Pathobiology, Faculty of Veterinary Medicine,University of Tabriz, Tabriz, Iran. Abresan square, 29th Bahman Blv. Postal code: 51666-14779. East Azerbayjan, Iran.

## INTRODUCTION

3-Methylindole (3-MI) is major ruminal fermentation product of tryptophan. It is one of the etiologic factors of acute bovine pulmonary edema and emphysema (ABPE) [12]. Pathologically, ABPE includes of pulmonary edema and congestion, interstitial emphysema, alveolar hyaline membrane formation, and hyperplasia of type II pneumocytes [6]. The substance also in goat [4], sheep [5], horse [25], rat [31] and mice [26] selectively induces damage of Clara cells and olfactory epithelium, the major site of cytochrome P-450-dependent microsomal mixed function oxidases [11]. Glutathione peroxidase concentration is inversely correlated with the severity of 3-MI-induced pulmonary lesions [16] by conjugation with produced free radicals [8]. In mice by injection of 3-MI dissolved in corn oil initial lesions has been observed in clara cells that were loss of microvilli and secretory granules followed by swelling of the endoplasmic reticulum and mitochondria [27]. It is determined 3-MI lead to hyperplasia and metaplasia with lamina proprial fibrosis and ossification in olfactory mucosal epithelium [18].

Usage of Cremophor has been associated with Histamine release, severe anaphylactoid hypersensitivity reactions, hyperlipidemia, abnormal lipoprotein patterns and aggregation of erythrocytes [15].

The usage of Propylene Glycol can cause renal insufficiency and hepatic dysfunction thus raises the risk of toxicity. Its toxic effects include hyper osmolality, metabolic acidosis, acute kidney injury, and a sepsis-like syndrome [23].

The aim of this study was to compare complications of 3-MI dissolved in two solvents, Cremophor and Propylene Glycol on BALB/c mice by histopathologic and stereologic studies.

## MATERIALS AND METHODS

*Animals*

Female Balb/C mice 56 days of age (23 - 27 g) were divided into 13 groups of eight and had access to food and water ad libitum. Mice were housed in plastic cages (4 per cage) with wire bar lids on bedding of sawdust in an air-conditioned room with 12 h light: dark cycles.

*Injection protocol*

After a 7-day acclimation period, for 5 groups, mice were injected with 400, 500, 600, 700 and 800 mg/kg from solution of 3-MI in propylene glycol and similarly for 5 other groups with Cremophor. As well as 2 groups were injected with Propylene glycol, Cremophor with the dosage that is necessary for solving the highest dose of 3-MI; one group received normal saline with the volume equal with Cremophor (drug vehicle) group. Briefly, 125 mg of 3-MI was dissolved per mL of sterile filtered Propylene glycol and 30 mg of 3-MI was dissolved per mL of sterile filtered Cremophor. Once injected intra peritoneally, the animals were monitored 3-4 h for external reaction signs from the injection.

*LD50 determination*

LD50 was determined with linear regression test using sigma plot version 10 software.

*Histology*

Mice were killed by cervical dislocation 48 h after 3-MI administration. If they were alive till the hour 48. The lungs were fixed in situ by tracheal perfusion with 10% phosphate buffered formalin. Other tissues were fixed by immersion in fixative. Paraffin sections of lungs were stained with hematoxylin and eosin (H&E) and examined by light microscopy. Bronchiolar lesions were graded as follows: 0 = no histologic lesion, + 1 = rare exfoliated epithelial cells, +2 = exfoliation, nuclear pyknosis, and cytoplasmic vacuolation of epithelium coexistent with histologically normal bronchioles, + 3 = multifocal epithelial necrosis and sloughing, and +4 = extensive epithelial necrosis and sloughing. The score was increased by 1 if lesions extended proximally into the axial bronchus [24].

Alveolar epithelialization was assessed by counting of type II pneumocytes in at least 100 alveoli per each microscopic slide. The highest volume of Propylene glycol and Cremophor given to any animal in the treatment groups that received 3-methylindole was 6.4 mL/kg and 26.6 mL/kg respectively.

Data were analyzed using SPSS version19. For typeII pneumocytes percentage analysis, one way ANOVA with Tukey as post hoc and for bronchiolar lesions Kruskal-wallis with Mann-whitney has been used.

## RESULTS

*LD50*

LD50 was 610.16 mg 3-MI /kg and 687.5 mg 3-MI /kg for Propylene glycol and Cremophor respectively (Figure 1).

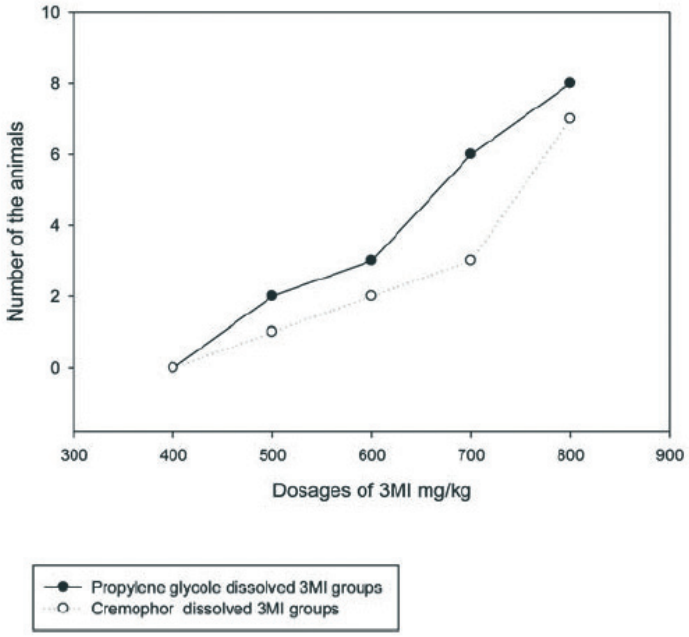


**Figure 1.** Linear diagram of LD50 determination.

*Clinical findings*

Increase in respiration rate, tachypnea and shallow respiration was observed in clinical examination.

*Necropsy findings*

Alveolar and bronchial hyperemia was significant in lungs. Some superficial wounds were observed in upper respiratory airways. Trachea and Hungarian airways were severely hyperemic. There were some debris's in airways canals. We couldn't find any lesion in other organs.

*Histopathology*

*Type II Pneumocytes*

There was a significant difference between Cremophor dissolved 3-MI groups and control group as discretely ($P < 0.001$) and so for Cremophor group as drug vehicle ($P < 0.001$ for doses higher than 400 mg/kg and $P = 0.027$ for the dose 400 mg/kg). Also Propylene glycol dissolved 3-MI dosage groups had considerable differences with control group in each dosage. Propylene glycol (drug vehicle) group showed substantial differences with Propylene glycol dissolved 3-MI groups ($P < 0.001$ for doses higher than 400 mg/kg ; $P = 0.004$ for the dose 400 mg/kg). The dose 800 mg/kg of Cremophor dissolved 3-MI showed noticeable difference with the dose 800 mg/kg of Propylene glycol dissolved 3-MI ($P = 0.009$). There were no significant differences between other groups statistically (Table 1).

*Bronchiolar scores*

There were significant differences between Cremophor dissolved 3-MI groups and control group as separately (for dosage 400 mg/kg $P = 0.009$, for dosage 500 mg/kg $P = 0.003$ and for other dosages $P < 0.001$). Propylene glycol dissolved 3-MI dosage groups had substantial differences with control group (for dosage 400 mg/kg $P = 0.003$, for other dosages $P < 0.001$). Propylene glycol (drug vehicle) group showed substantial differences with Propylene glycol dissolved 3-MI dosage groups except with the dosage 400 mg/kg (for dosage 500mg/kg $P = 0.007$, dosage 600 mg/kg $P = 0.002$, 700 & 800mg/kg $P < 0.001$). Cremophor (drug vehicle) group appeared to has significant differences with Cremophor dissolved 3-MI dosage groups except for the dose 400 mg/kg (for dosage 500 mg/kg $P = 0.045$, dosage 600 mg/kg $P = 0.002$, 700 and 800 mg/kg $P < 0.001$) [Table1].

Noticeable alveolar edema and emphysema, type I pneumocytes necrosis, severe epithelialization and raising of type II pneumocytes number were observed in both groups. Slight endothlial lesion, rarely degeneration of type II pneumocyte and raised neutrophil infiltration around vessels were observed in higher dosages of both groups (Figures 2, 3 and 4). Moderate interstitial edema, hyperemia and increase

**Table 1.** Quantitative and semi quantitative data of bronchiolar lesions and type II pneumocyte percentage changes in alveoli.

| Group | Dose: mg/kg | Alveolar type II Pneumocyte Percentage Mean+SD | Bronchiol lesion Score Mean+SD |
|---|---|---|---|
| Cremophor + 3-MI | 400 | 17.5 + 2.2 | 0.62 + 0.51 |
| | 500 | 22.5 + 4,07 | 0.87 + 0.64 |
| | 600 | 27.5 + 4.92 | 1.37 + 0.51 |
| | 700 | 36.00 + 5.13 | 2.12 + 0.83 |
| | 800 | 47.62 + 6.76 | 2.75 + 0.71 |
| Propylene glycol + 3-MI | 400 | 20.62 + 2.72 | 0.87 + 0.64 |
| | 500 | 26.72 + 2.71 | 1.25 + 0.46 |
| | 600 | 30.12 + 4.61 | 1.75 + 0.71 |
| | 700 | 38.5 + 5.34 | 2.5 + 0.93 |
| | 800 | 56.87 + 8.42 | 3.00 + 0.76 |
| Cremophor (mL) | 26.6 | 9.06 + 3.52 | 0.25 + 0.46 |
| Propylene glycol (mL) | 6.4 | 10.75 + 3.61 | 0.37 + 0.51 |
| Control (Normal saline) (mL) | 26.6 | 6.31 + 2.08 | 0.00 + 0.00 |

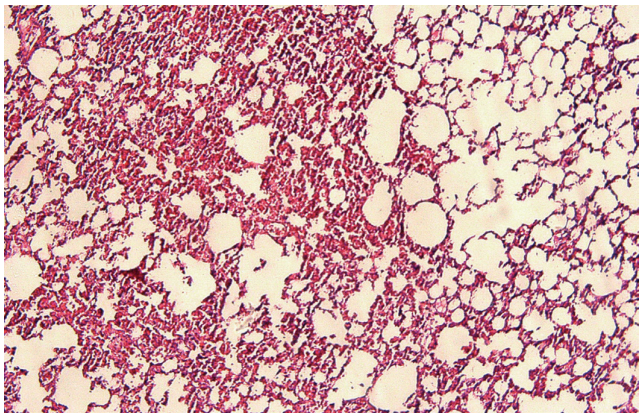

**Figure 2.** Marked epithelialization in the group 800 mg/kg 3-MI dissolved in Propylene glycol. H&E staining (Obj. 20x).

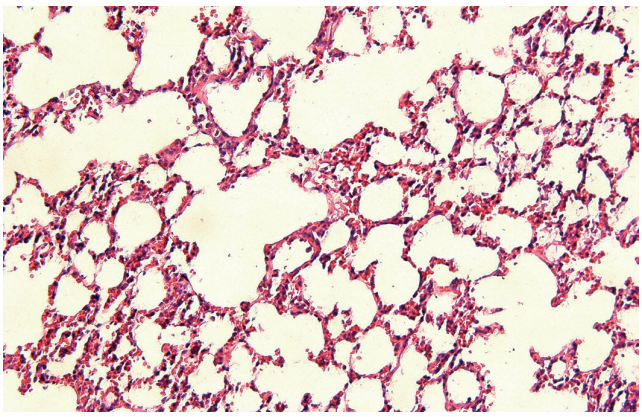

**Figure 3.** Emphysema and hyperemia in the group 800 mg/kg 3-MI dissolved in Propylene glycol. H&E staining (Obj. 40x).

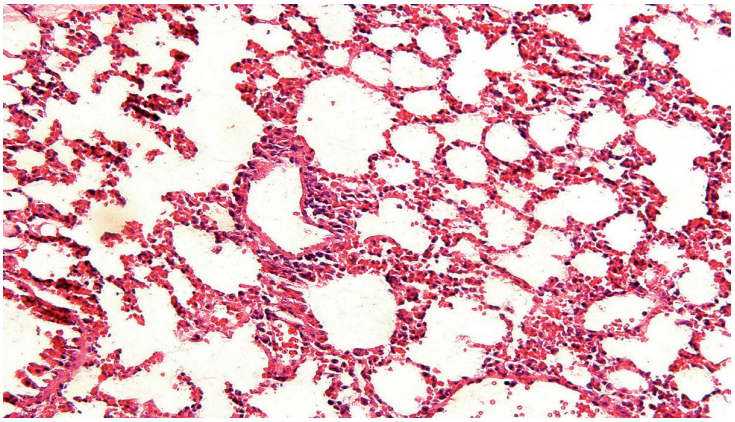

**Figure 4.** Slight perivascular infiltration of leukocytes in the group 800 mg/kg 3-MI dissolved in Cremophor. H&E staining (Obj. 40x).

of alveolar macrophages were observed too. Increasing of lymphocytes was noticed after the dose 600 mg/kg moderately on a dose dependent manner. There was a Hyaline membrane in the wall of some air sacs. Endothelial capillary damages in Cremophor dissolved 3-MI groups were slightly lower. Although damage to the vessels was not that much progressed in both groups. The type of alterations was as the same among both groups. But severity of the lesions was higher in Propylene glycol groups.

## DISCUSSION

The results of this research indicates that intraperitoneal infusion of 3-MI dissolved in Propylene glycol and Cremophor cause noticeable changes in the respiratory systems of mice. Effects of 3-MI in Propylene glycol is more severe than 3-MI dissolved in Cremophor but type of the lesions is not different. Bronchial lesions were moderate compare to the effects of 3-MI in ponies and horses [18].

Severity of vascular lesions in propylene glycol dissolved 3-MI was greater than groups that received the Cremophor dissolved 3-MI.

Data about administration of 3-MI in Cremophor EL to calves and goats were similar to those described here. In goats, the predominant target cells of 3-MI-induced pneumotoxicity are the nonciliated bronchiolar epithelial cells in airways and the Type I alveolar epithelial cells and capillary endothelial cells in alveolar parenchyma [4]. Similar target cells were observed in this study. These findings suggest that similar pathways may exist in the metabolism of 3-MI and that similar responses to 3-MI-induced lung injury exist in these species. Bronchiolar epithelium is the major target of 3-MI toxicosis in ponies [18].

According to our findings alveolar epithelial damage, pulmonary edema and airways damages were the major lesions in both groups. These outcomes are parallel with other researches [24]. We observed minor changes in vascular endothelium. These changes were observed in cattle too [1].

Type I epithelial cell necrosis is a consistent feature of 3-MI pneumotoxicity in ruminants [4] and mice but not in the horse [25]. Pulmonary capillary endothelial cells have been shown to contain cytochrome P-450 mixed-function oxidases in the rabbit [22] and in the rat that are required to metabolize 3-MI to an active electrophilic intermediate [9]. Type I epithelial cells are intimately associated with capillary endothelium and may be particularly sensitive to cytotoxicity because of their large membrane surface area and relative lack of organelles and endogenous detoxification mechanisms [31].

Type II cell hyperplasia seen in the former studies may indicate that type I cell necrosis had occurred. It must also be noted that rats in some former studies received dietary supplementation with vitamin E and selenium [14] which may have mitigated epithelial changes. Selenium-containing enzymes of the glutathione peroxidase-glutathione reductase system have been shown to play an important role in detoxification of 3-MI. Pulmonary glutathione modulation has demonstrated that 3-MI-induced pneumotoxicity can be increased or decreased by depletion with diethyl maleate or supplementation with cysteine, respectively [21].

This study indicated that toxicity of 3-MI dissolved in different solvents in mice has different severity from each other and toxicity of propylene glycol as drug vehicle is higher than Cremophor, although Cremophor is toxic too. Investigations have revealed that Cromophor, a widely used formulation vehicle, and a biologically and pharmacologically active ingredient of various commercially available drugs has side effects itself [10]. For example, when used in paclitaxel administrations causing important biological events depending on the dose and duration of infusion. Cremophor EL's toxicity is believed to be due to histamine release and increase in serum lipid concentration. Normal saline diluted with Cremophor is found to be toxic for myocytes [15]. But of course we couldn't find any alteration in vascular smooth muscle cells.

It has been shown in several studies that Propylene glycol can cause metabolic abnormalities. Moreover, Propylene glycol toxicity is an iatrogenic complication that can be life threatening [30]. It includes development of serum hyperosmolality, lactic acidosis, and kidney failure [2]. Proximal tubular necrosis is the cause of acute kidney injury from Propylene glycol [19].

We couldn't find any problem in kidney in our research Propylene glycol-induced intoxication can also mimic sepsis or systemic inflammatory response syndrome (SIRS) [30]. Severity of bronchiolar lesions is decreased by inducers of cytochrome P-448 or P-450 while inhibitors have no significant effect [24]. Significant differences in mortality or lesions between treatment groups of mice suggest that solvent is important in 3-MI toxicosis.

Several possibilities could account for this disparity. One possibility is that different isoenzymes of the cytochrome P-450 mixed-function oxidase that are responsible for the metabolic activation of each compound reside within the cell populations. There may be heterogeneity in the cellular distribution of the particular isoenzymes activating 3-MI. Another possibility is that the injured cell population is susceptible to toxic injury because it lacks well-developed detoxifying pathways. Other factors, such as cellular levels of protective substances, including glutathione and other protective nucleophilic compounds and lipid-soluble, low-molecular-weight free-radical scavengers may play a pivotal role in modulating cell toxicity after these xenobiotics are metabolized [3,7]

There is not any evidence of a role for glucocorticoids or mineralocorticoids in the pathogenesis of 3-MI-induced bronchiolitis [17]. Other study has been showed that olfactory impairment was highly correlated with the reduction of olfactory neuronal population assessed by olfactory protein marker expression in C57BL6 mice treated with 3-methylindole [13]. New report related that majority of the cell damages is due to CYP1A1 activity, and other cytochrome P450 enzymes may also contribute to 3-MI-mediated DNA damage [29]. 3-MI exposure has the potential to increase the rate of mutation within these lung cells and thus may act as a potential carcinogen [28]. Also BEAS-2B cells transfected with CYP2F1 and CYP3A4 were susceptible to 3-MI-mediated cytotoxicity in humans [20].

## CONCLUSION

Solution of 3-MI in Propylene glycol is more toxic than 3-MI in Cremophor. Although both of the solvents has toxicity but with the dosage necessary for solving 3-MI they couldn't produce important lesions in the body of mice.

SOURCES AND MANUFACTURERS

[1]Cremophor EL - C5135, Sigma-Aldrich, Milwaukee, WI, USA.

[2]Propylene glycol - P 4347, Sigma-Aldrich, Milwaukee, WI, USA.

[3]3-Methyle indole - M51458, Sigma-Aldrich, Milwaukee, WI, USA.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## REFERENCES

**1 Atwal O.S. & Persofsky M.S. 1984.** Ultrastructural pathology of intrapulmonary arteries in 3-methylindole-induced pneumotoxicity in cattle: II. Glycogen accumulation in the smooth muscle cells and intimal changes. *The Journal of Pathology*. 142(2): 141-149.

**2 Barnes B.J., Gerst C., Smith J.R., Terrell A.R. & Mullins M.E. 2006.** Osmolal gap as a surrogate marker for serum propylene glycol concentrations in patients receiving lorazepam for sedation. *Pharmacotherapy*. 26(1): 23-33.

**3 Boyd M.R., Stiko A., Statham C.N. & Jones R.B. 1982. P**rotective role of endogenous pulmonary glutathione and other sulfhydryl compounds against lung damage by alkylating agents: Investigations with 4-ipomeanol in the rat. *Biochemical Pharmacology*. 31(8): 1597-1583.

**4 Bradley B.J. & Carlson J.R. 1980.** Ultrastructural pulmonary changes induced by intravenously administered 3- methylindole in goats. *American Journal of Pathology*. 99(3): 551-559.

**5 Bradley B.J., Carlson J.R. & Dickinson E.O. 1978.** 3-Methylindole- induced pulmonary edema and emphysema in sheep. *American Journal of Veterinary Research*. 39(8): 1355-1358.

**6 Breeze R.G., Selman I.E., Pirie H.M. & Wiseman A.1978.** A reappraisal of atypical interstitial pneumonia in cattle. *Bovine Practitioner*. 13: 53-81.

**7 Burley F.E. & Bray T.M. 1983.** Effect of dietary vitamin A on the mixed function oxidases and the pneumotoxicity of 3- methylindole in goats. *Canadian Journal of Physiology and Pharmacology* . 61(8): 816-821.

**8 Carlson J.R., Nocerini M.R. & Breeze R.G. 1984.** The role of metabolism in 3-methylindole-induced acute lung injury. In: *Progress in Tryptophan and Serotonin Research*. Schlossberger H.G., Kochen W., Linzen B. & Steinhart H. (Eds). New York: Walter de Gruyter & Co., 483p.

**9 Carlson J.R. & Yost G.S. 1989.** 3-Methylindole acute lung injury resulting from ruminal fermentation of tryptophan. In: Cheeke P.R. (Ed). *Toxicants of Plant Origin*. Boca Raton: CRC Press, pp.107-123.

**10 Elderblom H., Verweij J., Nooter K. & Sparreboom A. 2001.** Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. *European Journal of Cancer*. 37(13): 1590-1598.

**11 Griffin K.A., Johnson C.B., Breger R.K. & Franklin R.B. 1982.** Effects of inducers and inhibitors of cytochrome P-450- linked monooxygenases on the toxicity, *in vitro* metabolism and *in vivo* irreversible binding of 2-methylnaphthalene in mice. *Journal of Pharmacology and Experimental Therapeutics*. 221(3): 517-524.

**12 Hammond A.C., Bradley B.J., Yokoyama M.T., Carlson J.R. & Dickinson E.O. 1979.** 3-Methylindole and naturally occurring acute bovine pulmonary edema and emphysema. *American Journal of Veterinary Research*. 40(10): 1398-1401.

**13 Kim J.W., Hong S.L., Lee C.H., Jeon E.H. & Choi A.R. 2010.** Relationship between olfactory function and olfactory neuronal population in C57BL6 mice injected intraperitoneally with 3-methylindole. *Otolaryngol Head Neck Surgery*. 143(6): 837-842.

**14 Kiorpes A.L., Keith I.M. & Dubielzig R.R. 1988.** Pulmonary changes in rats following administration of 3-methylindole in Cremophor EL. *Histology & Histopathology*. 3(2): 125-132.

**15 Lorenz W., Reimann H.J., Schmal A., Dormann P., Schwarz B., Neugebauer E. & Doenicke A. 1977.** Histamine release in dogs by Cremophor E1 and its derivatives: oxethylated oleic acid is the most effective constituent. *Agents Actions*. 7(1): 63-67.

**16 Menill J.C. & Bray T.M. 1983.** The effect of dietary and sulfur compounds in alleviating 3-methylindole-induced pulmonary toxicity in goats. *Journal of Nutrition*. 113(9): 1725-1731.

**17 Miller M.A., Kottler S.J., Ramos-Vara J.A., Johnson P.J., Ganjam V.K. & Evans T.J. 2003.** 3-methylindole induces transient olfactory mucosal injury in ponies. *Veterinary Pathology*. 40(4): 363-702.

**18 Miller M.A. & O'Bryan M.A. 2003.** Ultrastructural changes and olfactory deficits during 3-methylindole-induced olfactory mucosal necrosis and repair in mice. *Ultrastructural Pathology*. 27(1): 13-21.

**19 Morshed K.M., Jain S.K. & McMartin K.E. 1998.** Propylene glycol-mediated cell injury in a primary culture of human proximal tubule cells. *Toxicological Sciences*. 46(2): 410-417.

**20 Nichols W.K., Mehta R., Skordos K., Macé K., Pfeifer A.M., Carr B.A., Minko T., Burchiel S.W. & Yost G.S. 2003.** 3-methylindole-induced toxicity to human bronchial epithelial cell lines. *Toxicological Sciences*. 71(2): 229-363.

**21 Nocerini M.R., Carlson J.R. & Breeze R.G. 1983.** Effect of glutathione status on covalent binding and pneumotoxicity of 3-methylindole in goats. *Life Sciences*. 32(5): 449-458.

**22 Serabjit-Singh C.J., Nishio S.J., Philpot R.M. & Plopper C.G. 1988.** The distribution of cytochrome P-450 monoxygenase in cells of rabbit lung: An ultrastructural immunocytochemical characterization. *Molecular Pharmacology*. 33(3): 279-289.

**23 Tausif Z., Charles G. & Mark A.P. 2007.** Recognition, Treatment and Prevention of Propylene Glycol Toxicity. *Seminars in Dialysis*. 20(3): 217-219.

**24 Turk M.A.M., Flory W. & Henk W.G. 1986.** Chemical Modulation of 3-Methylindole Toxicosis in Mice: Effect on Bronchiolar and Olfactory Mucosal injury. *Veterinary Pathology*. 23(5): 563-570.

**25 Turk M.A., Breeze R.G. & Gallina A.M. 1983.** Pathologic changes in 3-methylindole-induced equine bronchiolitis. *American Journal of Pathology*. 110(2): 209-218.

**26 Turk M.A.M., Flory W. & Henk W.G. 1984.** Dose response in 3-methylindole-induced bronchiolar epithelial necrosis in mice. *Research Communications in Chemical Pathology & Pharmacology*. 46(3): 351-362.

**27 Turk. M.A., Henk. W.G & Flory W. 1987.** 3-Methylindole-induced nasal mucosal damage in mice. *Veterinary Pathology*. 24(5): 400-403.

**28 Weems J.M., Cutler N.S., Moore C., Nichols W.K., Martin D., Makin E., Lamb J.G. & Yost G.S. 2009.** 3-Methylindole is Mutagenic and a Possible Pulmonary Carcinogen. *Toxicological Sciences*. 112(1): 59-67.

**29 Weems J.M. & G.S. Yost G.S. 2010.** 3-Methylindole Metabolites Induce Lung CYP1A1 and CYP2F1 Enzymes by AhR and Non-AhR Mechanisms, Respectively. *Chemical Research in Toxicology*. 23(3): 696-704.

**30 WilsonK.C., Reardon C., Theodore A.C. & Farber H.W. 2005.** Propylene glycol toxicity: a severe iatrogenic illness in ICU patients receiving IV benzodiazepines. A case series and prospective, observational pilot study. *Chest*. 128(3): 1674-1681.

**31 Woods L.W., Wilson D.W., Schiedt M.J. & Giri S.N. 1993.** Structural and biochemical changes in lungs of 3-methylindoletreated rats. *American Journal of Pathology*. 142(1): 129-138.