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Research Article

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Establishment of Acceptable Daily Intakes (ADIs) and Risk Assessment for Ephedrine, Menichlopholan, Anacolin, and Etisazole Hydrochloride

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Abstract

BACKGROUND: Prior to implementing a positive list system (PLS), there is a need to establish acceptable daily intake (ADI) and maximum residue limit (MRL) for veterinary drugs that have been approved a few decades ago in South Korea. On top of that, chronic dietary exposure assessment of veterinary drug residues should be performed to determine whether the use of these veterinary drugs would cause health concerns or not.

METHODS AND RESULTS: To establish the ADI, the relevant toxicological data were collected from evaluation reports issued by international organizations. A slightly modified global estimate of chronic dietary exposure (GECDE) model was employed in the exposure assessment owing to the limited residual data. Therefore, only the ADI of ephedrine was established due to insufficient data for the other veterinary drugs. Thus, instead of ADI, the threshold of toxicological concern (TTC) value was used for the other drugs. Lastly, the hazard index (HI) was calculated, except for etizazole hydrochloride, due to the potential of mutagenicity.

CONCLUSION(S): The HI values of ephedrine, menichlopholan, and anacolin were found to be as high as 6.4%, suggesting that chronic dietary exposure to the residues from these uses was unlikely to be a public health concern. Further research for exposure assessment of veterinary drug residues should be performed using up-to-date Korean national health and nutrition examination survey (KNHANES) food consumption data. In addition, all relevant available data sources should be utilized for identifying the potentials of toxicity.

Key words: Anacolin, Ephedrine, Etisazole hydrochloride, Menichlopholan, Risk assessment

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Introduction

Veterinary drugs have been used to prevent disease-outbreak from animals and enhance performance [1]. Additionally, the sales of veterinary drugs have been annually rising in worldwide along with an in-

crease in supply and consumption of livestock products (i.e. meats and eggs) [2,3].

However, abuse or misuse of veterinary drug to animals could lead to human health concern because their residues might present in food. For example, the contaminated food issue called as 'fipronil-case' would be well-known [4]. Fipronil-case is caused by a use of illegal pesticide to egg and egg products in Europe. Fipronil is authorized to be used as veterinary drug to treat mites and ticks in pets like dogs and cats, although it is not permitted to be intended for food producing animals such as chicken in Europe [4].

To enhance the regulation of pesticide or veterinary drug residues in food, the positive list system (PLS) has been already implementing in many countries such as Europe, USA and Japan. PLS indicates that pesticide, feed additives or veterinary drugs, which have been not permitted in domestics, should be applied to 0.01 mg/kg, close to the value for limit of quantitation (LOQ).

In South Korea, several veterinary drugs (i.e. ephedrine, menichlopholan, anacolin and etisazole hydrochloride) have been approved in the past without setting MRLs and HBGVs [i.e., acceptable daily intake (ADI)].

Ephedrine has long been used in humans to prevent and treat both bronchitis and asthma [5]. According to the FDA document, its effects have been known to include increased blood pressure, by activating alpha- and beta-adrenergic receptors, and increased cardiac contractility.

Menichlopholan, one of the halogen phenols, is used for the treatment of *Fasciola hepatica* in ruminants, which noted in the specification of bilebon injection. *Fasociola hepatica* is a common species of medically important trematodes in Korean cattle [6].

Anacolin is an anticholinergic drug used for the prevention and treatment of acute indigestion in cattle, pigs, and horses, as described in veterinary drug specifications. A mode of action of anticholinergics is to compete with acetylcholine for cholinergic receptors and act mainly through the muscarinic acetylcholine receptors in the parasympathetic nervous system [7].

In the Ectimar® specification, etisazole hydrochloride is a broad-spectrum fungicide used to control trichophytosis and microsporosis in cattle, swine, and horses. Since some fungicides could have induced the hazardous effects such as genotoxic or teratogenic effects [8,9], it is required to review the other toxicological aspects of etisazole hydrochloride, thoroughly.

To respond the PLS of veterinary drugs, effective to be January 1st 2024, there is a need to evaluate the most appropriate ADI and MRL for four veterinary drugs as mentioned above. Therefore, the aims of this study were to establish the acceptable daily intake of these compounds based on risk assessment by reviewing their safety evaluation documents and calculating the hazard index (HI).

Materials and Methods

Hazard identification

Toxicological data were collected from evaluation reports issued by several international organizations, including the Center for Drug Evaluation Research, European Food Safety Agency.

Determination of point of departure (POD)

The most sensitive endpoint was determined by comparing the toxicological/pharmacological data.

Exposure assessment

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has used the GECDE model, which it first proposed in 2009, for dietary exposure assessment of veterinary drug residue evaluation since 2017. This model may be more practical than the previous model diet, the theoretical maximum daily intake (TMDI), since it allows simultaneous consideration of both high and general consumers. The global estimate of chronic dietary exposure (GECDE) model calculates the sum of the highest dietary exposure for a food category, based on high (97.5th percentile) consumption levels, plus the mean dietary exposure for all other food categories, using individual countries' food consumption data for the general population. This study used the estimate of chronic dietary exposure (ECDE) model because no median residue-level data were available. The original GECDE model (a) and ECDE model (b) calculation equations are as follow:

$$\frac{\begin{pmatrix}\text{high dietary exposure for one food}\\ \text{97.5th percentile by consumers} \times\\ \text{median residue levels}\end{pmatrix} + \begin{pmatrix}\text{mean dietary exposure for all other foods}\\ \text{average consumption by general population} \times\\ \text{median residue levels}\end{pmatrix}}{\text{body weight (kg)}}$$

(a) GECDE model equation

$$\frac{\begin{array}{l}high\ dietary\ exposusre\ for\ one\ food \\ \left(97.5th\ percentile\ by\ consumers \right. \\ \left.\times\ proposed\ MRLs\right)\end{array} + \begin{array}{l}mean\ dietary\ exposure\ for\ all\ other\ foods \\ \left(average\ consumption\ by\ general\ population\right. \\ \left.\times\ proposed\ MRLs\right)\end{array}}{body\ weight\ (kg)}$$

(b) ECDE model equation

Threshold of Toxicological Concern (TTC) concept

The TTC approach was first introduced in 2016 by the European Food Safety Agency (EFSA) and World Health Organization (WHO) [10]. The TTC approach allows the prioritization of chemicals in the regulatory context to be determined, as a screening tool, because it provides toxicological reference values depending on the chemical's specific hazardous potential. Accordingly, this study superseded TTC values where data to evaluate the ADI were insufficient.

Food consumption data and Proposed MRLs

This study's exposure assessment incorporated the 2010-2016 KNHANES food consumption data provided by the Korean Disease Control and Prevention Agency (KDCA) and MRLs proposed by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) and the Ministry of Food and Drug Safety (MFDS).

Risk characterization

The HI was calculated using the following formula, where exposures below the threshold values, veterinary drug residues are unlikely to be a public health concern.

$$Hazard\ Index\ (\%) = \frac{Human\ daily\ exposure\ (mg/kg\ bw/day)}{ADI\ \text{or}\ TTC\ value(mg/kg\ bw/day)} \times 100$$

bw, body weight

Hazard identification of the target chemicals

To establish the ADI for the target chemicals, pharmacokinetic data (laboratory animals or humans); single- and repeated-dose toxicity data, and reproductive/developmental toxicity data; genotoxicity, carcinogenicity, clinical, and pharmacological (cardiovascular system, respiratory system, and central nervous system) data; and residue depletion trials data were investigated through Pubmed, PubChem and EPA comptox. From the investigation, the toxicological and pharmacological information of each chemical were summarized. In case of the chemical that had insufficient toxicological data to evaluate the ADI, TTC value determined to Cramer's decision tree was assigned.

Exposure and Risk assessment

Chronic dietary exposure to the target chemicals was estimated in this study. Finally, the HI was calculated by dividing the estimates into ADI or TTC values.

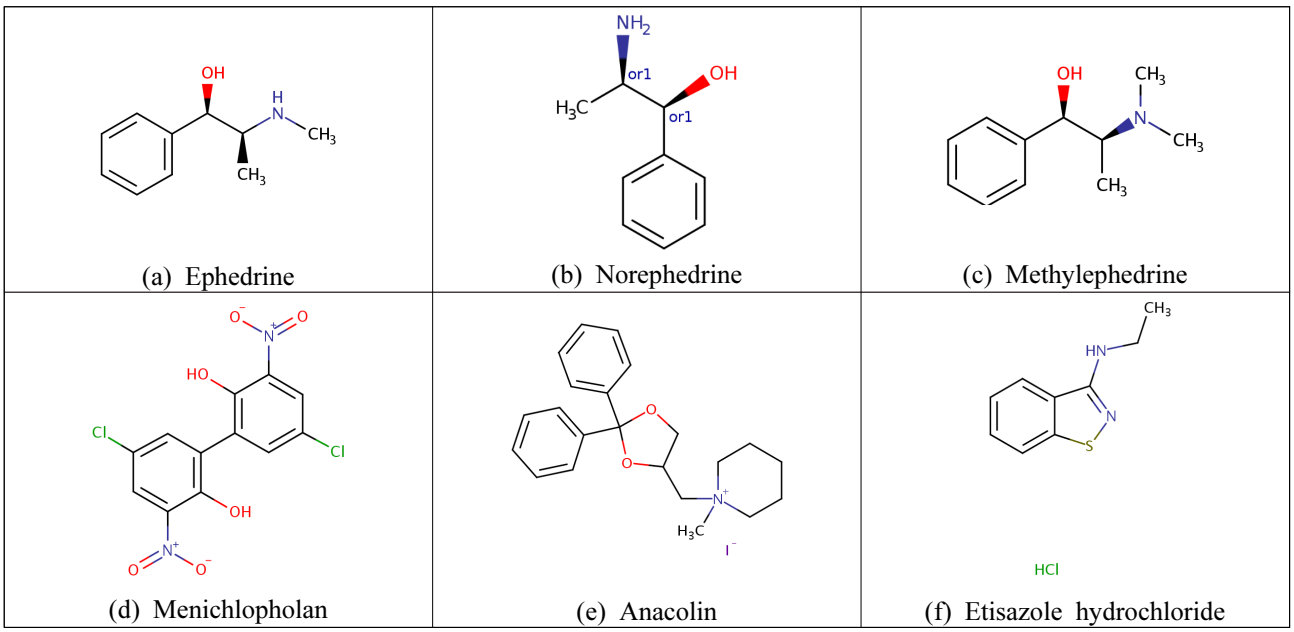

Fig. 1. Structures of ephedrine and its analogues, mechlopholan, anacolin, and etisazole hydrochloride

Results and Discussion

Hazard identification

The structures of all target chemicals, structural identifiers, and physicochemical characteristics are summarized in Fig. 1 and Tables 1 and 2.

Hazard identification of Ephedrine

Summary of ADME

Pharmacokinetic profiles are likely to differ depending on species. When various species are orally administered ephedrine, the parent compound is metabolized into norephedrine, hydroxy-norephedrine, and hydroxy-norephedrine [11]. When rabbits or humans are orally exposed to norephedrine, a hydroxy-norephedrine is produced [12]. These metabolites are produced via various metabolic pathways including aromatic hydroxylation, N-dealkylation, and deamination [13]. Little fecal excretion has been reported of these metabolites and ephedrine; therefore, the major route of excretion is likely urine [12]. In rats and humans, predominantly ephedrine is found in the urine, while norephedrine has mostly been noted in the urine of rabbits, dogs, and guinea pigs [13].

General toxicity

The lethal doses (LD_{50}) were investigated for multiple species and routes pertaining to ephedrine, ephedrine sulfate, or hydrochloride. Detailed general toxicity studies are provided in SI Table1.

Table 1. Physicochemical characteristics of ephedrine and its analogues

Generic name	Ephedrine	Norephedrine	Methylephedrine
IUPAC name	(1R,2S)-1-Hydroxy-2-(methylamino)-1-phenylpropane	(1R,2S)-2-Amino-1-phenyl-1-propanol	(1R,2S)-2-Dimethylamino-1-phenyl-1-propanol
Molecular formula	$C_{10}H_{15}NO$	$C_9H_{13}NO$	$C_{11}H_{17}NO$
CAS no.	299-42-3	492-41-1	552-79-4
Molecular weight	165.236 g/mol	151.210 g/mol	179.260 g/mol
Appearance	Colorless or slightly off-white liquid		
Vapor pressure	$2.61x10^{-3}$ - $2.76x10^{-3}$ mmHg	$1.10x10^{-3}$ - $2.76x10^{-3}$ mmHg	$2.56x10^{-3}$ - $1.96x10^{-2}$ mmHg
Melting point	40.5-90.4℃	101℃	21.2-87.6℃
Density	1.01-1.02 g/ml	1.07 g/ml	0.974-1.01 g/ml
Water solubility	$7.08x10^{-2}$ - 11.4 mol/L	0.132-11.0 mol/L	$2.98x10^{-2}$ - 10.9 mol/L
logKow	0.68-1.49	0.73	1.67

Table 2. Physicochemical characteristics of menichlopholan, anacolin, and etisazole hydrochloride

Generic name	Menichlopholan	Anacolin	Etisazole hydrochloride
IUPAC name	5,5'-Dichloro-3,3'-dinitro[1,1'-biphenyl]-2,2'-diol	1-[(2,2-Diphenyl-1,3-dioxolan-4-yl)methyl]-1-methylpiperidin-1-ium iodide	N-Ethyl-1,2-benzothiazol-3-amine--hydrogen chloride (1/1)
Molecular formula	$C_{12}H_6C_{l2}N_2O_6$	$C_{22}H_{28}INO_2$	$C_9H_{11}ClN_2S$
CAS no.	10331-57-4	21216-78-4	7716-59-8
Molecular weight	345.09 g/mol	465.4 g/mol	214.71 g/mol
Appearance	Colorless or yellow liquid		
Vapor pressure	$9.15x10^{-11}$ - $8.03x10^{-8}$ mmHg	3.28 - 10^{-8} mmHg	$3.02x10^{-6}$ - $3.44x10^{-2}$ mmHg
Melting point	191-214℃	204℃	71.6-136℃
Density	1.73-1.74 g/ml	-	1.23-1.25 g/ml
Water solubility	$1.32x10^{-6}$ - $1.88x10^{-5}$ mol/L	$4.20x10^{-7}$ - 7.0 mol/L	$5.19x10^{-4}$ - 7.13 mol/L
logKow	3.23-4.88	0.9	-0.60-2.85

SI Table 1. Summary of the general toxicology of ephedrine

Study/ Duration		Species	Compounds	Route/ Concentration	Results	References
Single admin.	-	Mice (B6C3F1)	Ephedrine sulfate	PO/-	LD_{50} 1072 mg/kg bw (F); 812 mg/kg bw (M)	NTP, 1986
	-	Rat (Fisher 344)	Ephedrine sulfate	PO/-	All exposure groups died	NTP, 1986
	-	Rat (Fisher 344)	Ephedrine hydrochloride and caffeine	PO/-	Similar prevalence of clinical signs btw single and mixture groups	Dunnick et al., 2007
	-	Mice (NMRI)	Ephedrine hydrochloride	IV/-	LD_{50} 74 mg/kg bw (M); Tarchycardia, convulsions and aggression/hyperkinesia	Marvola, 1976
	-	Rat	Ephedrine sulfate	IV/-	LD_{50} 102 mg/kg bw (M); Convulsions, hypernea and paralysis	Graham and Kuizenga, 1948
	-	Rat	Ephedrine sulfate	IV/-	LD_{50} ≥135 mg/kg bw; dead after injection 10 min. later and reporting clinical signs, such as anxiety and skin irritation etc. before dead	Chen et al., 1926
	-	Rabbit	Ephedrine sulfate	IV/-	LD_{50} ≥66 mg/kg bw; Clinical signs (anxiety, incoordination, hyperpnea, restlessness etc.)	Chen et al., 1926
	-	Rabbit	L-ephedrine	IV/-	LD_{50} 60 mg/kg bw; no information about toxic effects	Warren and Werner, 1946
	-	Rabbit	Ephedrine sulfate	IV/-	LD_{50} 73 mg/kg bw; Clinical signs (convulsions, increased respiration and paralysis followed by prostration)	Graham and Kuizenga, 1948
	-	Dog	Ephedrine sulfate	IV/-	LD_{50} ≥70 mg/kg bw; Clinical signs (convulsions, tremor, incoordination, increased respiration)	Chen et al., 1926
Repeated admin.	13 week	Mice (B6C3F1)	Ephedrine sulfate	PO (feeding); 310-5000 ppm (47-750 mg/kg bw/day)	≥75 mg/kg bw/day: no histopathological changes related to the compound, body weight gain loss; ≥150 mg/kg bw/day: hyperactivity, excitability etc.	NTP, 1986
	13 week	Rat (Fisher 344)	Ephedrine sulfate	PO (feeding); 125-2000 ppm (12.5-200 mg/kg bw/day)	≥50 mg/kg bw/day: no histopathological changes related to the compound, body weight gain loss; ≥100 mg/kg bw/day: hyperactivity, excitability	NTP, 1986, ECHA
Local tolerance	-	Rabbit	Ephedrine sulfate	IV/ ≥ 16.6 mg/kg	thrombosis	Chen et al., 1926

* PO, per oral; IV, intravenous

Mouse

A study of ephedrine sulfate in mice, carried out by the National Toxicological Program (NTP), reported an LD_{50} of 1072 mg/kg bw for females and 812 mg/kg bw for males, after a single oral administration. For intravenous administration of ephedrine hydrochloride, the LD_{50} was 74 mg/kg bw for males [14].

Rat

The LD_{50} values of ephedrine sulfate were evaluated in rats. A single-dose toxicity study carried out by the NTP in the 1980s revealed that all exposure groups died, making it impossible to calculate the lethal dose. A later single-dose toxicity study, using intravenous administration, resulted in a male LD_{50} of 102 mg/kg bw [15]. Another study reported an LD_{50} greater than 135 mg/kg bw via intravenous administration in rats [16].

Rabbit and dog

The LD_{50} values for rabbits have been reported as 60 or greater than 63 mg/kg bw [16,17]. In dogs, the LD_{50} for a single intravenous administration of ephedrine sulfate was greater than 70 mg/kg bw [16]. A

cross these animals, clinical signs after ephedrine administration included convulsions and changes in respiration.

Repeated-dose toxicity

The 13-week repeated-dose toxicity studies in rodents (rats and mice) conducted by the European Chemical Harmonization Agency (ECHA) and NTP reported no compound-related pathological changes; however, decreased weight gain was observed in all rats or mice treated with 50 or 75 mg/kg bw/day, respectively. Hyperactivity and excitement due to drug administration were observed in the groups treated with more than 100 or 150 mg/kg bw/day, rats and mice, respectively. Therefore, the NOAEL of ephedrine in the 13-week repeated-dose toxicity study in rats and mice was determined to be 22 and 35 mg/kg bw/day, respectively.

Local tolerance test

In a local tolerance test using rabbits, the administration of ephedrine sulfate (5% solution, 50 mg/ml) induced thrombosis. However, there is a lack of clinical relevance regarding this effect due to the absence of a clinical report [16]. Detailed study information is presented in SI Table 1.

Reproductive and developmental toxicity

Through the available reproductive and developmental toxicity documents pertaining to Ephedrine and its analogues, the no-adverse effect level (NOAEL) of Ephedrine was considered 4 mg/kg bw/day, equivalent to 10 mg/kg bw/day of ephedrine sulfate. In this paragraph, we briefly introduce reproductive and

SI Table 2. Summary of reproductive and developmental toxicology of ephedrine

Species	Compounds	Route/ Concentration	Study duration	Results	References
Chick embryos (stage 19)	Ephedrine and caffeine	0, 0.5, 5 μmol (0, 1, 10 mg/eggs)	3 days (observation at day 14)	≥0.5 μmol : cardiovascular malformation 0.5 μmol : cardiac malformation rate (8%); 5 μmol : cardiac malformation rate (26%), all cases were reported to small ventricular septal defects	Nishikawa et al., 1985
Chick embryos	L-ephedrine	0.5, 1, 5, 10, 20 μmol/egg	4 days	≥1 μmol/egg : increase of malformation 20 μmol/egg: reduction of embryo survival	Nishikawa et al., 1985
Chick embryos	L-ephedrine	14 μmol/egg	4 days	Aortic arch abnormalities	Nishikawa et al., 1985
Rat (Wistar)	Ephedrine hydrochloride	(IP) 0.1, 1, 10, 50 mg/kg	GD 9-11 (observation at day 20)	Cardiovascular malformation rate of the fetuses was 20.5 % and the frequency of the malformation was dose-dependent; All reported malformation were ventricular septal defects and two cases were with the overriding aorta; No difference in malformation rate according to the gestation period	Kanai et al., 1986
Rat	Ephedrine sulfate	0, 2, 10, 60 mg/kg	Male, before mating day 28 - GD; female, before mating day 14 - GD 7	No evidence of drug-related effects of early life developmental stages (fertilization and embryonic stages)	FDA, 2021
Rat	Ephedrine sulfate	10, 60 mg/kg	GD 6-17	Fetal toxicity NOAEL 10 mg/kg; 60 mg/kg: decreased fetus survival and weight loss, abnormal head motion	FDA, 2021
Rabbit	Ephedrine sulfate	(IV) ~ 20 mg/kg	GD 6-20	No evidence of malformation or embryo-fetus toxicity	FDA, 2021
Rat	Ephedrine sulfate	(IV) ~ 60 mg/kg	GD 6-lactation period day 20	Fetal toxicity NOAEL 10 mg/kg; 60 mg/kg: decreased fetus survival rate and weight loss accompanied by the increased maternal mortality	FDA, 2021
Juvenile Rat	Ephedrine sulfate	(IV) 2, 10, 60 mg/kg	PND 35-56	Developmental NOAEL 10 mg/kg; 60 mg/kg: mortality increased	FDA, 2021

* IP, intraperitoneal; IV, intravenous; GD, gestation day; PND, Postnatal day

developmental toxicity data from the FDA REZIPRES® approval (2021). Other studies discussing the reproductive and developmental toxicity of ephedrine are summarized in SI Table 2.

The effect of fertility and early developmental stage was not observed by ephedrine sulfate in rats. From available data, no effects were reported when male rats were exposed to 0, 2, 10, or 60 mg/kg ephedrine sulfate for both 28 days prior to mating and through gestation, and females were treated for 14 days prior to mating through gestational day 7. However, decreased fetal body weights were observed when pregnant rats were administered intravenous bolus doses of 60 mg/kg ephedrine sulfate from gestational days 6 to 17. The dose was also related to evidence of maternal toxicity, such as decreased body weight and abnormal head movements. However, fetal body weight was unaffected at 10 mg/kg. Additionally, in a study in which pregnant rabbits were administered an intravenous bolus dose of up to 20 mg/kg ephedrine sulfate daily from gestational day 6 to lactation day 20, there was no evidence of malformations or embryo/fetal toxicity. However, the high dose of 20 mg/kg ephedrine sulfate was considered related to pharmacological maternal effects, such as increased respiration rate, dilated pupils, and piloerection. Additionally, when juvenile rats were intravenously exposed to 2, 10, or 60 mg/kg bw/day ephedrine sulfate from postnatal day (PND) 35 to 56, adverse effects—an increased mortality incidence—were only reported for the 60 mg/kg bw/day dose. Therefore, the no-adverse-effect level was considered to be 10 mg/kg bw/day ephedrine sulfate. Lastly, in a study in which pregnant rats were administered an intravenous bolus dose of up to 60 mg/kg ephedrine sulfate daily from gestational day 6 to lactation day 20, decreased fetal survival and body weight, linked with maternal toxicity, was noted at a 60 mg/kg. No adverse effects were observed at a dose of 10 mg/kg.

Genotoxicity

A variety of *in vitro* and *in vivo* genotoxicity studies have been conducted. The *in vitro* bacterial reverse mutation test (Ames test); *in vitro* DNA damage tests using human lymphocytes, Chinese hamster ovary cells (CHO), or rat hepatocytes; and *in vitro* mutation tests using mouse lymphoma [17-22]. From the *in vivo* genotoxicity test, the FDA concluded that ephedrine seems to be a non-genotoxic chemical, as all ephedrine sulfate-treated groups were reported to be negative in the micronuclei test. Detailed explanations of the *in vitro*/*in vivo* genotoxicity studies are given in SI Table 3.

SI Table 3. Summary of genetic toxicology of ephedrine

Test/assay	Compounds	Subjects	Tested concentration	Results	References
In vitro bacterial reverse mutation test (Ames assay)	Ephedrine sulfate	*Salmonella typhimurium* (TA 97, TA 98, TA 100, TA 1535)	0, 100, 333, 1000, 3333, 10000 μg/plate (with/without S9 metabolic activation)	negative	NTP, 1986; Zeiger et al., 1988; EFSA, 2013
In vitro DNA damage test (comet assay)	(-)-Ephedrine	Human peripheral lymphocytes	0, 0.0005, 0.001, 0.01, 0.2, 1, 5, 50, 150, 350, 500 μM	negative	Radakovic et al., 2011
In vitro DNA damage test (SCE, sister chromatid exchange assay)	Ephedrine sulfate	Chinese hamster ovary cells (CHO)	0, 5600, 6000, 6400, 7000, 7600, 8000 μg/mL with S9 (2 hours treatment); 0, 1490, 1740, 1990, 2490, 2760, 3000 μg/ml without S9 (22-24 hours treatment)	equivocal	NTP, 1986
In vitro Alkaline elution assay	Ephedrine sulfate	Rat hepatocytes (from male SD rats)	3, 7, 10 mM	negative	Storer et al., 1996
In vitro Mouse Lymphoma assay	(-)-Ephedrine	Mice lymphoma cells (L5178Y tk (+/-) 3.7.2C)	0, 1.5, 4.5, 15, 45, 150, 450 μg/mL (without S9 metabolic activation)	negative	McGregor et al., 1988
In vitro Chromosome aberration assay	Ephedrine sulfate	Chinese hamster ovary cells (CHO)	0, 6, 8, 10 mM (with/without S9 metabolic activation)	negative	Hilliard et al., 1988
In vivo micronucleus assay	Ephedrine sulfate	Rat bone marrow	No information	negative	FDA, 2021

Carcinogenicity

The NTP investigated the carcinogenicity of ephedrine by administering 0, 19 or 37.5 mg/kg bw/day of ephedrine to mice for 103 weeks. The results showed decreased average body weight in each sex, but no evidence of potential carcinogenic effects. Thus, the NOAEL was determined to 37.5 mg/kg bw/day. A similar study in rats administered 0, 6.25, or 12.5 mg/kg bw/day of ephedrine for 103 weeks. Again, NOAEL was designated as the highest concentration. Similarly, according to the FDA document (2021), when rats and mice were exposed to ephedrine sulfate up to 10 and 27 mg/kg bw/day, respectively, ephedrine did not induce tumors. Therefore, the FDA authors concluded that it would not be a carcinogen, and no adverse effects were determined at 10 mg/kg and 27 mg/kg bw/day in rats and mice, respectively. Accounting for all of the available data, shown in SI Table 4, it was concluded that there was no evidence of carcinogenicity in ephedrine.

Clinical observation

A summary of clinical studies is shown in SI Table 5. These results indicate that ephedrine and norephe-

SI Table 4. Summary of carcinogenicity of ephedrine

Test duration	Species	Compounds	Route/concentration	Results	References
103 weeks	Mice (B6C3F1)	Ephedrine	PO (feeding); 0, 125, 250 ppm (0, 19, 37.5 mg/kg bw/day)	Decrease in average weight in each sex; No evidence of carcinogen	NTP, 1986
103 weeks	Rat (Fisher 344)	Ephedrine	PO (feeding); 0, 125, 250 ppm (0, 6.25, 12.5 mg/kg bw/day)	Decrease in average weight in each sex; No evidence of carcinogen	NTP, 1986
2-years	Mice, rat	Ephedrine sulfate	Up to 10/27 mg/kg bw/day (rat/mice)	No evidence of carcinogen	FDA, 2021

* PO, per oral

SI Table 5. Summary of clinical studies of ephedrine

Compounds	Subjects	Duration/Concentration	Results	References
Ephedrine sulfate	Pregnant	-	No impact on major birth defects, miscarriage, or adverse maternal/fetus effects associated with drug administration	FDA, 2021
Norephedrine	Healthy volunteers (6 men)	(-)-norephedrine 37.5 mg, (+)-norephedrine 37.5 mg, (±)-norephedrine 75 mg	Cardiovascular LOAEL 37.5 mg/day; Compared to before administration, (±) and (-)-norephedrine groups significantly increased systolic/diastolic blood pressure; No difference in before and after blood pressure in (+)-norephedrine group	Stockley et al., 1994
Ephedrine	180 overweight patients	24 weeks (3 times a day)/ Four groups: placebo, ephedrine/ caffeine combination (20/200 mg), caffeine 200 mg, and ephedrine 20 mg	Side-effects were reported; common symptom was insomnia and followed by tremors (17 and 9 patients, respectively); No significant changes of systolic/diastolic blood pressure and heart rate	Astrup et al., 1992
Ephedrine	16 health volunteers	PO 50 mg; intranasal 5 or 10 mg	Systolic/diastolic blood pressure and heart rate were increased in dose-dependent manner; 5 mg intranasal group: no difference of placebo group	Berlin et al., 2001
Ephedrine	Health volunteers (5 men and 3 women)	PO 0.25, 0.5, 1.0 mg/kg	Change in heart rate was linearly related to plasma ephedrine concentration within the observed range of concentration under 200 μg/L	Persky et al., 2004
Ephedrine	32 overweight children	20 weeks (3 times a day)/ <80 kg (100 mg ephedrine + 10 mg caffeine); >80 kg (200 mg ephedrine + 20 mg caffeine)	No difference btw placebo and treatment groups of the heart rate, blood pressure or subjective side-effects	Molnar et al., 2000

drine were likely to be associated with cardiovascular effects. Specifically, norephedrine induced a change in the diastolic and systolic blood pressure in six healthy males who took 37.5 mg ephedrine; thus, the lowest observed adverse effect level (LOAEL) was determined to be 37.5 mg/person/day [27]. A cross-sectional study also reported an increase in diastolic/systolic blood pressure associated with ephedrine administration [23].

Pharmacology

Similarly, cardiovascular effects have also been observed in laboratory animals. For instance, a study of L-ephedrine in SD male rats reported changes in both systemic and pulmonary blood pressure, concluding that ephedrine-related changes are mediated by the stimulation of direct alpha-adrenergic receptors and controlled by beta-adrenergic receptors [24]. The effects of ephedrine on the central nervous system (CNS) were investigated in mice and rats. In particular, when mice were exposed to ephedrine, motor activity markedly increased after 3 h [25]. Regarding respiratory effects, the increased respiration rate per

SI Table 6. Summary of pharmacology of ephedrine

Test	Species	Compounds/route/conc.	Results	References
Cardio-vascular	Rat (SD male)	L-ephedrine; (IV) 1, 3, 10, 30 mg/kg	Increase in systemic and pulmonary pressure with dose-dependent manner in single or cumulative dose; increased systemic arterial pressure at 10 mg/kg; increased systemic pressure and decreased pulmonary pressure by pre-treatment phentolamine (0.5 mg/kg, IV) in conscious rat; no alteration of increased systemic arterial pressure by pre-treatment reserpine alone or AMPT combination; therefore, the alteration of systemic and pulmonary pressure via ephedrine treatment can be mediated directive alpha adrenal receptors stimulation and controlled by beta adrenal receptors	CDER, 2016; Liles et al., 2006
	Dogs, Cats	(Both, IV) D(-)-ephedrine 0.33 mg/kg, L(+)-ephedrine 0.99 mg/kg, L(+)-pseudoephedrine 1.65 mg/kg; (Dogs, IV) D(-)-pseudoephedrine 0.33, 3.3, 9.9, 16.5 mg/kg; (Cats, IV) D(-)-pseudoephedrine 0.33, 3.3, 6.6, 13.2, 26.4 mg/kg *pre-treatment in atropine sulfate 1 mg/kg	(all animals, D(-)-ephedrine 0.33 mg/kg and L(+)-ephedrine 0.99 mg/kg, L(+)-pseudoephedrine 1.65 mg/kg) pressor effects; (dog D(-)-pseudoephedrine 0.33~16.5 mg/kg and cat D(-)-pseudoephedrine 0.33~26.4 mg/kg) depressor effects, duration of the observed effects and severity was dose-dependent; (dog, D(-)-ephedrine, L(+)-ephedrine, L(+)-pseudoephedrine) heart rate peaked; (dog, D(-)-pseudoephedrine) increased heart rate in dose-dependent manner; especially, peaked at 9.9 mg/kg	CDER, 2016; Patil et al., 1965
	Dog (male)	Ephedrine sulfate; (IV) 5 mg/kg	After treatment 13~63 min., decrease in heart rate; without recovery to baseline over the whole tested period	CDER, 2016; Graham & Kuizenga et al., 1948
Respiration	Dog (male)	Ephedrine sulfate; (IV) 5 or 100 mg/kg	After treatment 13~63 min., increase in respiratory rate per min.; without recovery to baseline over the whole tested period	CDER, 2016; Graham & Kuizenga et al., 1948
Central nervous system	Mice (NMRI male)	(-)-ephedrine; (IV) 40 mg/kg	After treatment 3 hours later, significantly increase in the locomotor activity	CDER, 2016; Marvola & Kivirinta et al., 1978
	Rat	(-)-ephedrine; (IV) 0, 9.9, 19.8, 39.6, 79.2 mg/kg	Beam interruptions remarkably increased at ≥ 19.8 mg/kg and peaked at 39.6 mg/kg when evaluating locomotor activity using photocell activity cage based on the calculation of the total number of beam interruptions within 40 min.	CDER, 2016; Meng et al., 1999

* IV, intravenous

minute is remarkable in male dogs [15]. The pharmacological aspects of ephedrine are presented in SI Table 6.

POD for Ephedrine

To determine the most sensitive point of departure for ephedrine, the relevant points of departure were summarized (SI Table 7). In order to establish HBGV, human data are preferred as the uncertainties resulted from animal data could be eliminated [26]. Therefore, the clinical data for norephedrine could be a candidate for point of departure of ephedrine [27]. The authors figured out that the lowest observed adverse effect level (LOAEL) of norephedrine for cardiovascular effects was 37.5 mg/person/day [27]. However, ephedrine has considered to be transformed to norephedrine approximately 13.2% in human [28]. Due to the fact that this clinical study has several disadvantages

SI Table 7. Summary of point of departures (PODs) of ephedrine

Test/assays		Compounds	Results	References
Repeated toxicity	Rat 13 weeks	Ephedrine sulfate	NOAEL 35/22 mg/kg bw/day (female/male)	NTP, 1986, ECHA
	Mice 13 weeks	Ephedrine sulfate	NOAEL 75 mg/kg bw/day	NTP, 1986,
Reproductive and developmental toxicity	Chicks embryo	L-ephedrine	No derived NOAEL due to the dead of all treatment groups	Nishikawa et al., 1985
	Chicks embryo	L-ephedrine	Fetal developmental toxicity NOAEL 0.5 μmol/egg	Nishikawa et al., 1985
	Chicks embryo	L-ephedrine	No derived NOAEL because of the aortic arch abnormalities	Nishikawa et al., 1985
	Rat	Ephedrine hydrochloride	No derived NOAEL owing to the occurrence of the cardiovascular defects in all treatment groups	Kanai et al., 1986
	Rat (Segment I)	Ephedrine sulfate	Embryonic/fetal developmental toxicity NOAEL 60 mg/kg bw/day; no evidence of malformation	FDA, 2021
	Rat (Segment II)	Ephedrine sulfate	Embryonic/fetal developmental toxicity NOAEL 10 mg/kg bw/day; no evidence of malformation	FDA, 2021
	Rat (Segment II)	Ephedrine sulfate	Embryonic/fetal developmental toxicity NOAEL 20 mg/kg bw/day; no evidence of malformation	FDA, 2021
	Rat (Segment III)	Ephedrine sulfate	Pre/postnatal development and maternal toxicity NOAEL 10 mg/kg bw/day	FDA, 2021
	Rat (Juvenile)	Ephedrine sulfate	Developmental toxicity NOAEL 10 mg/kg bw/day	FDA, 2021
Genotoxicity	Ames test	Ephedrine sulfate	Negative	NTP, 1986; Zeiger et al., 1988
	Comet test	(-)-Ephedrine	Negative	Radakovic et al., 2011
	SCE test	Ephedrine sulfate	Equivocal	NTP, 1986
	DNA elution test	Ephedrine sulfate	Negative	Storer et al., 1996
	Mutation test	(-)-Ephedrine	Negative	McGregor et al., 1988
	Chromosome aberration test	Ephedrine sulfate	Negative	Hilliard et al., 1988
	Micronucleus test	Ephedrine sulfate	Negative	FDA, 2021
Carcinogenicity	Mice 103 weeks	Ephedrine	NOAEL 37.5 mg/kg bw/day; no evidence of carcinogen	NTP, 1986
	Rat 103 weeks	Ephedrine	NOAEL 12.5 mg/kg bw/day; no evidence of carcinogen	NTP, 1986
	Mice and rat two-years	Ephedrine sulfate	NOAEL 10/27 mg/kg bw/day (rat/mice); no evidence of carcinogen	FDA, 2021
Clinical studies	Cardiovascular	Norephedrine	LOAEL 37.5 mg/persone/day	EFSA, 2013

Table 3. Rationales for setting acceptable daily intake (ADI) of ephedrine

ADI	0.04 mg/kg bw/day
Toxicity study	Reproductive and developmental toxicity study
Compound	Ephedrine sulfate
Species	Rat
Route	IV (intravenous)
Duration	Gestation day 6 ~ lactation day 20
Adverse effects	Maternal toxicity, decrease in fetal survival and weight gain
NOAEL	4 mg/kg bw/day (as a ephedrine)
Uncertainty factor	100 (difference of the intra/inter species)
References	FDA, 2021

(i.e., a limited number of subjects, single-administration), it is reasonable that animal data for ephedrine hydrochloride or sulfate would be good candidates. Consequently, the appropriate NOAEL was determined to be 4 mg/kg bw/day ephedrine based on fetal and maternal toxicity in rats intravenously exposed to ephedrine sulfate. The observed developmental toxicity is considered to the most sensitive effect resulted from the exposure to ephedrine and its analogues this is because the adverse effect was shown during early life stages. A default uncertainty factor of 100 was applied to the NOAEL to adjust for the intra/inter species difference, resulting in a final ADI of 0.04 mg/kg bw/day (Table 3).

Hazard identification of Menichlopholan

Menichlopholan belongs to the halogen phenols, which include nitroxynyl, disophenol, and bithionol [29]. Menichlopholan has been reported to have moderate-to-severe acute toxicity. For example, the oral LD_{50} for menichlopholan in rats and hamsters has been reported to be 10 and 50 mg/kg bw, respectively, according to PubChem. Contrastingly, the LD_{50} range for bithionol was noted as between 7-760 mg/kg bw when rats and mice were exposed through multiple routes (oral, intraperitoneal, and intravenous), as reported in PubChem. However, the LD_{50} for nitroxynil was 125 mg/kg bw in mammals and ranged from 170 to 450 mg/kg bw in rats, mice, and dogs, as outlined in the evaluation report of the European Medicine Agency (EMA). The lethal dose values for hexachlorphene has been established in rats via a variety of routes: 56 mg/kg bw, 22 mg/kg bw, 7.5 mg/kg bw, and 340 mg/m3 for oral, intraperitoneal, intravenous, and inhalation, respectively, as mentioned in the MSD (Merck&Co.) and TCI America Inc. hexachlorphene safety data sheets (SDS). The acute toxicity data for the halogen phenols are shown in SI Table 8. Based on these data, the acute toxicity of menichlopholan was higher than that of other halogen phenols. Except for the acute toxicity studies, no other toxicity data were available.

Hazard identification of Anacolin

Although the acute toxicity data of anacolin are not available in detail (SI Table 8), compound-related

SI Table 8. Summary of acute toxicity data of halogen phenols and anacolin

Species	Compounds	Route/concentration	Results	References
Rat	Menichlopholan	PO/-	LD_{50} 10 mg/kg bw	PubChem
Hamster	Menichlopholan	PO/-	LD_{50} 50 mg/kg bw	PubChem
Goat and sheep	Menichlopholan	PO/-	LDLo 15 mg/kg bw	PubChem
Mammals	Nitroxynil	PO/-	LDLo 125 mg/kg bw	EMA, 1998
Rat, mouse, dogs	Nitroxynil	PO/-	LD_{50} 170-450 mg/kg bw	EMA, 1998
Rat	Bithionol	PO/-	LD_{50} 7 mg/kg bw	PubChem
Mouse	Bithionol	PO/-	LD_{50} 760 mg/kg bw	PubChem
Mouse	Bithionol	IP/-	LD_{50} 100 mg/kg bw	PubChem
Mouse	Bithionol	IV/-	LD_{50} 18 mg/kg bw	PubChem
Rat	Anacolin	PO/-	LD_{50} 1820 mg/kg bw	PubChem
Rat	Anacolin	IP/-	LD_{50} 713 mg/kg bw	PubChem
Mouse	Anacolin	IP/-	LD_{50} 46.9 mg/kg bw; parasympathetic inhibition	PubChem
mouse	Anacolin	SC/-	LD_{50} 713 mg/mg bw	PubChem

* PO, per oral; IP, intraperitoneal; IV, intravenous; SC, subcutaneous; LDLo, lowest lethal dose

Table 4. Rationales for allocating threshold of toxicological concern (TTC) values of menichlopholan, anacolin, and etisazole Hydrochloride

TTC category	Values
Genotoxic compounds with structural alerts	0.0025 μg/kg bw/day
Carbamates and organophosphates	0.0003 mg/kg bw/day
Cramer's Class III	0.0015 mg/kg bw/day
Cramer's Class II	0.0090 mg/kg bw/day
Cramer's Class I	0.030 mg/kg bw/day
Rationale	Menichlopholan and Anacolin have structurally more than one phenyl ring, so they include TTC category of Cramer's Class III
References	EFSA and WHO, 2016

effects—such as parasympathetic blockade—were reported in the Polish Journal of Pharmacology and Pharmacy at 1978. However, no other toxicity information (i.e., mutagenesis, carcinogenesis, and reproductive/developmental toxicity) was available.

POD for menichlopholan and anacolin

Due to the absence of adequate toxicity data for establishing the ADI of menichlopholan and anacolin, the TTC concept was used to evaluate the toxicological reference dose, which provides toxicological threshold values for the structural class (Table 4). In this study, menichlopholan and anacolin were assigned as 0.0015 mg/kg bw/day of Cramer's class III, as both chemicals have more than one phenyl or benzene ring.

Hazard identification of etisazole hydrochloride

To establish the ADI for this drug, safety documents were investigated for information such as ADME, general toxicity, and reproductive and developmental toxicity, but relevant data were not available.

However, clinical findings have been reported in humans. When Ectimar® containing 10% etisazole was applied to a mid-fifties farmer's wrist at least twice a day, severe contact dermatitis was observed after a few days [30]. Moreover, the signs persisted for three weeks despite corticosteroid therapy. The patch test, performed after the rash was resolved, was positive for etizazole 48 and 96 h later. Another similar clinical case reported an allergy to Ectimar® [31]. Presumably, the fragile S-N binding in etisazole could be easily broken in contact with the skin, indicating that metabolites could not cause hypersensitive reactions [30]. This hypothesis, however, seems limited by the lack of relevant published pharmacokinetic research. However, the genotoxic potential of this drug was confirmed by the QSAR database (ECHA); etisazole has been predicted to be a mutagen in both CAESAR mutagenicity model and SARPY mutagenicity model in VEGA (Q)SAR platform as a good reliability. On top of that, it has been also predicted as non-easily biodegradable in Danish QSAR database, which indicating that etisazole has the potentials of persistency in the environment. Therefore, further studies on ADME, genotoxicity and residue depletion trials are warranted to ensure the safety of this chemical.

SI Table 9. Chronic dietary exposure estimates of ephedrine

Food	Proposed MRLs	MR/TR ratio	Converted TR value	Intake (kg/day)		Exposure (mg/day)	
				Average	High	Average	High
Cattle muscle	0.01	1	0.01	0.0220	0.2623	0.0002	0.0026
Porcine muscle	0.01	1	0.01	0.0471	0.4319	0.0005	0.0043
Equine muscle	0.01	1	0.01	<0.0001	<0.0001	<0.0001	<0.0001
Sheep muscle	0.01	1	0.01	<0.0001	0.001	<0.0001	<0.0001
Goat muscle	0.01	1	0.01	<0.0001	0.2184	<0.0001	0.0022
Milk	0.005	1	0.005	0.0963	0.5533	0.0005	0.0028
Total amount of exposure (mg/day)						0.0050	
ADI = 0.04 mg/kg bw/day x 60 kg						2.4	
HI (%)						0.2	

SI Table 10. Chronic dietary exposure estimates of menichlopholan

Food	Proposed MRLs	MR/TR ratio	Converted TR value	Intake (kg/day)		Exposure (mg/day)	
				Average	High	Average	High
Cattle muscle	0.01	1	0.01	0.0220	0.2623	0.0002	0.0026
Milk	0.01	1	0.01	0.0963	0.5533	0.0010	0.0055
Total amount of exposure (mg/day)						0.0058	
ADI = 0.0015 mg/kg bw/day x 60 kg						0.09	
HI (%)						6.4	

SI Table 11. Chronic dietary exposure estimates of anacolin

Food	Proposed MRLs	MR/TR ratio	Converted TR value	Intake (kg/day)		Exposure (mg/day)	
				Average	High	Average	High
Cattle muscle	0.01	1	0.01	0.0220	0.2623	0.0002	0.0026
Porcine muscle	0.01	1	0.01	0.0471	0.4319	0.0005	0.0043
Milk	0.01	1	0.01	<0.0001	<0.0001	<0.0001	<0.0001
Total amount of exposure (mg/day)						0.0045	
ADI = 0.0015 mg/kg bw/day x 60 kg						0.09	
HI (%)						5.0	

POD for etisazole hydrochloride

Using the TTC concept, the TTC value assigned to etisazole hydrochloride (0.0025 μg/kg bw/day) was assigned as presented in Table 4, until there was no evidence of mutagen, because etizazole was predicted to be a 'suspected mutagen'.

Exposure and risk assessment

This study estimated chronic dietary exposure to all the tested chemicals. Detailed information regarding the exposure assessment for each drug is provided in SI Tables 9 to 11. Finally, the HI was calculated by dividing the estimates into ADI or TTC values. The exposure, toxicological reference doses, and risks are summarized in Table 5; risk ranged from 0.2 to 6.4%, except for etizazole hydrochloride. It is essential that further studies ensure the overall safety of etizazole hydrochloride. Taking everything into account, ephedrine, menichlopholan, and anacolin do not present any risk to the consumer, and the proposed MRLs are likely to be appropriate to protect public health.

Note

The authors declare no conflict of interest.

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Table 5. Summary of risk assessment

Therapeutic classification	Compounds	Residue definition	ADI or TTC value (mg/day)	Exposure (mg/day)	HI (%)
Not allocated	Ephedrine	Ephedrine	2.4	0.0050	0.2
Anthelmintic	Menichlopholan	Menichlopholan	0.009	0.0050	6.4
Anticholinergic agent	Anacolin	Anacolin	0.009	0.0045	5.0
Antifungal	Etisazole hydrochloride	Etisazole hydrochloride	0.00015	0.0062	-

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