1,2,4-Benzenetricarboxylic acid, mixed decyl and octyl triesters (MDOT)

SUMMARY

1,2,4-Benzenetricarboxylic acid, mixed decyl and octyl triesters (MDOT or the "PMN Substance") is a large molecule of variable size chain composition (see table 1). It is intended to be used as a substitute for the diester phthalate plasticizers such as diethylhexyl phthalate (DEHP), certain of which are well-known experimental testicular toxins in the rat model, with the fetus being the most sensitive stage. Multiple characteristics of the triesters versus the diesters, along with experimental results on some of these triesters (subacute and subchronic studies, prenatal developmental toxicity studies, and reproduction/developmental toxicity screening tests) make it unlikely that they would share male reproductive system toxicity as exhibited by the diesters. In addition to negative study results, the triesters are metabolized quite differently than the diesters, making a shared mechanism even more unlikely. The mechanism by which DEHP causes testicular mal-development in the male rat is related to regulation of genes involved in steroidogenesis. A representative triester, trioctylbenzene-1,2,4-tricarboxylate (TOTM) did not repress these same genes in the fetal rat testis, as demonstrated by microarray technology, further arguing against a shared toxicological effect.

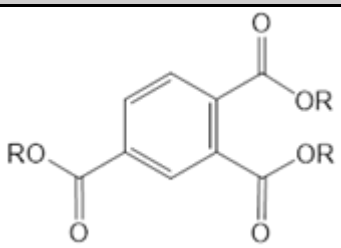
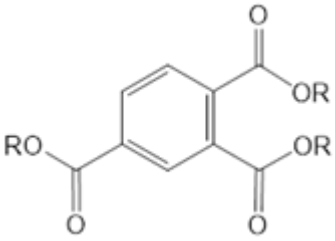
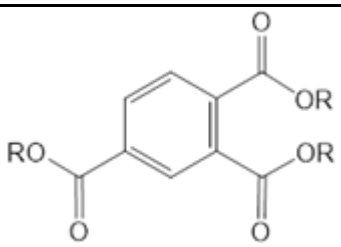
INTRODUCTION

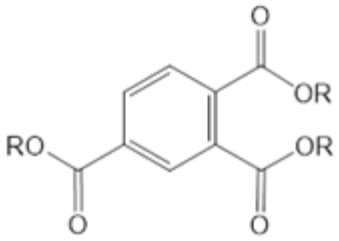
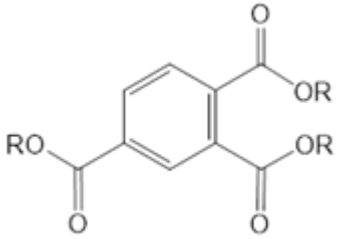
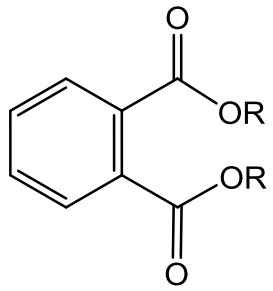
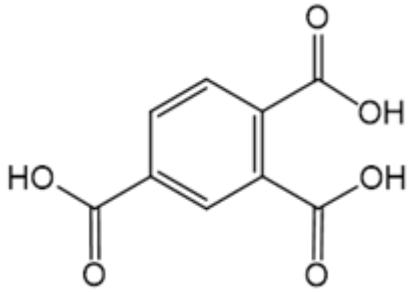
The PMN substance, 1,2,4-Benzenetricarboxylic acid, mixed decyl and octyl triesters (MDOT), is a member of a group of chemicals referred to as the trimellitates. As triesters of trimellitic acid, these chemicals share the same chemical trimellitic acid backbone of 1,2,4-benzene tricarboxylic acid, but vary with respect to length and branching of side-chains (See Table 1). The trimellitates are large, hydrophobic molecules with very low vapor pressure. While there is limited physical/chemical and toxicological data on the individual members of the group, read-across is appropriate in some circumstances, since the individual trimellitates are expected to behave similarly (both chemically and toxicologically), and available studies have borne this out. MDOT is considered a UVCB, i.e. a Chemical Substance of Unknown or Variable Composition, Complex Reaction Products and Biological Materials, in that it is not a single chemical structure, but rather a mixture of structural isomers whose side chains esters are linear decyl and octyl groups.

BTIT and TTDT (refer to Table 1 for full names and structures) are somewhat larger molecules than the PMN substance MDOT, whereas TOTM and TEHT overlap with the low molecular weight end of MDOT with respect to structural similarity.

Phthalates are related structures, though these chemicals are *diesters* of 1,2-benzenedicarboxylic acid. Bis(2-ethylhexyl) phthalate (DEHP) is the most widely studied of the phthalates and has shown developmental and reproductive effects following oral exposure in mice and rats.

TABLE 1: Referenced Chemicals

Chemical Name	Abbreviation	CAS No.	Structure
1,2,4-Benzenetricarboxylic acid, mixed decyl and octyl triesters	MDOT	CAS No. 90218-76-1 / 67989-23-5 EC No. 290-754-9 / 268-007-3	 Where R = $-(CH_2)_7CH_3$ $-(CH_2)_9CH_3$ Mixture of 8 structural isomers
Read across structures (trimellitates):			
Trioctylbenzene-1,2,4-tricarboxylate; trioctyl trimellitate	TOTM	CAS No. 89-04-3 EC No. 201-877-4	 Where R = $-(CH_2)_7CH_3$ 1 structure
Tris(2-ethylhexyl)benzene-1,2,4-tricarboxylate; Tris(2-ethylhexyl) trimellitate	TEHT	CAS No. 3319-31-1	 Where R = $-CH_2CH(Et)(Bu)$ 1 structure

1,2,4-Benzenetricarboxylic acid, tritridecyl ester; Tristridecyl trimellitate	TTDT	CAS No. 94109-09-8	 Where R = $-(CH_2)_{12}CH_3$ 1 structure
1,2,4-Benzenetricarboxylic acid, mixed branched tridecyl and isodecyl esters; Tridecyl trimellitate	BTIT	CAS No. 70225-05-7	 Where R = $-(CH_2)_{10}CH(CH_3)_2$ $-(CH_2)_7CH(CH_3)_2$ Mixture of 8 structural isomers
Related chemicals:			
Diethylhexyl phthalate	DEHP	CAS No. 84-66-2	 Where R = $-CH_2CH(Et)(Bu)$
Trimellitic Acid	TMLA	CAS No. 528-44-9	

SUMMARY OF TOXICOKINETICS OF TRIMELLITATES AND PHTHALATES

→ Triesters of trimellitic acid, including TEHT and TOTM (those most similar to the PMN substance, MDOT) and the somewhat larger triesters, TTDT and BTIT, appear to have the same low bioavailability, and are likely to share toxicokinetic characteristics.

→ The majority of TEHT, a triester structurally similar to the PMN substance, is excreted unchanged in the feces, whereas DEHP, a smaller diester, is excreted mainly in the urine as end-products of metabolism.

→ Dermal exposure to the larger trimellitates is expected to be quite low, and presumably below 1%. Exposure to the trimellitates through inhalation is expected to be negligible given the low vapor pressure of these chemicals.

TEHT was studied in *in-vitro* to determine ability to be hydrolyzed by rat intestinal hydrogenates (CIR-1984 Eastman Kodak). There was no indication of hydrolysis in this study, and no 2 ethylhexanol was released. The absorption, metabolism and excretion of this same chemical was studied *in-vivo* in fasted male Sprague-Dawley rats following oral administration (Cosmetic Information Review, 2015). Rats were given a single gavage dose of 100 mg/kg/bw ^{14}C-labeled TEHT in corn oil. Over a 144-hr time period following dosing, urine, feces and expired air were collected at various time intervals, after which time, the animals were killed and organs collected for analysis. Of the original dose, 94.4% of radioactivity was recovered, with the majority of the dose being excreted unchanged in the feces. The radiolabel was recovered as shown in Table 2. The overall absorption, metabolism and excretion of TEHT in this same study is shown graphically in the CIR (Cosmetic Information Review, 2015) (see Appendix 1).

TABLE 2: Radiolabel recovery following exposure of rat to 14C-labeled TEHT

14C recovered in:	Percentage recovered (average %):	Recovered as:	Notes
Expired air	1.9%	CO_2	Peak rates at 2-3 h and 8-12 h post-dosing
Urine	16%	mono-(2-ethylhexyl) trimellitate 2-ethylhexanol 2-ethylhexanoic acid 2-heptanone	
Feces	Ca. 75%	85% unchanged TEHT 1% mono-(2-ethylhexyl) trimellitate 7% di-(2-ethylhexyl) trimellitate unidentified polar metabolites	
Tissues	<0.6%		Greatest amounts in liver and adipose tissue

Toxicokinetic properties of the diester DEHP have been studied extensively, particularly following oral administration (European Chemicals Agency (ECHA) Information on Chemicals, 2011). Unlike the PMN substance, DEHP is rapidly absorbed following ingestion and inhalation, though exposure to internal organs is not to the intact diester, but rather to products resulting from rapid hydrolysis (Albro, 1986).

Both ester groups of DEHP can be hydrolyzed following oral exposure, leaving phthalic acid as a product (Kluwe, 1982). The first group is enzymatically hydrolyzed more readily than the second group, while only the liver is capable of hydrolyzing DEHP to phthalic acid. In fact, very small amounts of DEHP are hydrolyzed completely to phthalic acid. Over 90% of lower oral doses of DEHP (2000 ppm) are excreted in the urine, indicating a high degree of intestinal absorption. The initial hydrolysis product mono(2ethylhexyl) phthalate (MEHP) is rapidly absorbed from the gut and is conjugated to more polar glucuronide metabolites in many species, including man, while the rat is unable to form glucuronide conjugates. While initial hydrolysis products, MEHP and alcohol (2ethylhexanol (2-EH)), are further metabolized extensively *in-vivo* in rodents, these hydrolysis products are unoxidized or minimally oxidized in primates, including humans. Despite certain metabolic differences, excretion of polar metabolites via the urine is the main route of phthalate diester elimination in both rodent and man.

Comparing the toxicokinetic properties of diethylhexyl (DEHP) versus the triethylhexyl (TEHT) carboxylic acids, it is noteworthy that DEHP is excreted mainly in the urine as end-products of metabolism, whereas the majority of TEHT is excreted unchanged in the feces. Complete hydrolysis of the triesters to trimellitic acid was not demonstrated in the toxicokinetic study with TEHT though a certain degree of hydrolysis may be expected based on recovery products from the TEHT toxicokinetic study.

No biologically relevant dermal absorption of TEHT has been shown in *in-vitro* studies. One would expect dermal absorption of larger trimellitates to be lower than that of the smaller comparable phthalates. The relationship of alkyl side chain length to extent of *in-vivo* percutaneous absorption of phthalate diesters has been investigated (Alaa E. Elsisi, 1989). Percentage of dose eliminated in urine and feces along with dose remaining in the body (excluding actual exposure area) was considered indicative of amount of percutaneous absorption. In general, as the length of the alkyl side chain increased, the extent of absorption decreased, with dermal absorption being the greatest for chain lengths of C2 and C4. In addition, as the length of the side chain increased, the percentage of dose excreted in feces versus urine increased, indicating less metabolism of the larger molecules. Thus the same pattern of urinary versus fecal excretion seen following oral exposure has been demonstrated following dermal exposure. While degree of lipophilicity may be predicted to increase dermal absorption, this did not hold true for the phthalates above a certain molecule size. The largest of the phthalates tested, diisodecyl phthalate, was the most slowly excreted, with only 0.5% of the applied dose excreted in 7 days. Based on these results, exposure to the larger trimellitates through dermal absorption is expected to be quite low, and presumably below 1%. Exposure to the trimellitates through inhalation is expected to be negligible given the low vapor pressure of these chemicals.

BIOLOGICAL ACTIVITY OF BENZENE TRICARBOXYLIC ACID TRIESTERS VERSUS BENZENE DICARBOXYLIC ACID DIESTERS

→ An adverse effect on testicular development and function in the rat that is seen following exposure to certain phthalates is referred to as testicular mal-development (TMD). Phthalates have been shown to have testosterone-reducing effects due to effects on steroid synthesis following reduction in mRNA coding for target genes involved in testosterone synthesis.

→ Unlike DEHP, the triester TOTM, a close analog to the PMN substance, did not repress any of the TMD target genes extracted from fetal rat testis, indicating that the substance is unlikely to cause testicular dysgenesis in rats as is seen with some phthalate esters including DEHP.

→ In a subchronic feeding study on the PMN substance MDOT itself, no pathological changes in spermatogenesis or estrus cycle irregularities were seen in evaluation of the male and female reproductive systems. Microscopic and macroscopic observations of reproductive organs did not indicate any adverse effects on the reproductive organs (European Chemicals Agency (ECHA) Information on Chemicals, 2010).

→ Unlike the situation with the phthalates, studies with various mellitates and with trimellitic acid itself, have shown no effects on fertility or reproductive function in exposed rats or in their offspring.

In that DEHP, a diester of 1,2-benzenedicarboxylic acid, has been shown to disrupt the development of the male reproductive tract, i.e. have an anti-androgenic effects in male rodent studies, and given that triesters of benzenedicarboxylic acid are intended to be used as substitutes for DEHP, it is of interest to compare the two chemistries with respect to biological activity.

DEHP has been shown to adversely affect testicular development and function in male rats, with the fetus being the most sensitive to this effect (see Table 3). Other phthalates in addition to DEHP, a known experimental testicular toxin, have also been shown to affect fetal testosterone production with accompanying effects on testicular histopathology (Boberg, n.d.). The testosterone-reducing effects of DEHP and other diester phthalates like DIBP (diisobutyl phthalate) and DINP (diisononyl phthalate) are related to effects on steroid synthesis. An involvement of peroxisome proliferator-activated receptors (PPARs) has been proposed in the down regulation of testosterone production following phthalate exposure. Factors involved in testosterone synthesis ((cytochrome P450 side chain cleavage (P450scc), steroidogenic acute regulatory protein (STAR), cytochrome P450c17 (CYP17) and PPARγ)) are reduced following exposure of the rodent fetus to phthalates, including DEHP and DIBP. Corresponding mRNA genes coding for these and other factors related to steroid synthesis have been shown to be reduced following phthalate exposure in fetal rats ((Boberg, n.d.). This adverse effect on testicular development and function in the rat is referred to as testicular mal-development (TMD).

Histopathological changes in reproductive organs have not been seen following subchronic oral exposure of rats and dogs, or subchronic inhalation exposure in rats to trimellitic acid (TMLA) (OECD HPV, 2002). Further, trimellitic anhydride was not found to be teratogenic or fetotoxic in developmental toxicity studies in guinea pigs and rats; this finding is presumed to applicable to TMLA as well, since trimellitic anhydride is rapidly hydrolyzed to TMLA *in-vivo* (OECD HPV, 2002).] Certain triesters have been studied in subchronic feeding studies and in reproductive/developmental studies (see Appendix 2 for summary of studies). In a subchronic feeding study on MDOT itself, no pathological changes in spermatogenesis or estrus cycle irregularities were seen in evaluation of the male and female reproductive systems. Microscopic and macroscopic observations of reproductive organs did not indicate any adverse effects on the reproductive organs.

The closely related chemical, TOTM, was studied in a combined repeated dose toxicity / reproductive/developmental study [cited in: (Cosmetic Information Review, 2015)]. There was no effect of TOTM exposure (gavage up to 500 mg/kg/day) on reproductive function at any dose in either male or female rats despite some signs of general toxicity at the highest dose. A reproductive/developmental toxicity screening study was conducted on TEHT (European Chemicals Agency (ECHA) Information on Chemicals). There was a slight reduction in number of spermatocytes and spermatids 300 and 1000 mg/kg/day (see study description in and results in Appendix 2). There was no effect on reproductive ability in males or females, nor was there any effect on the offspring. A decrease in spermatocytes and spermatids as seen in the parental generation in the reproductive/developmental screening study was *not* seen in a subchronic 90-day study (exposure twice as long) with exposure to the same highest dose (1000 mg/kg/day) of TEHT analog, TOTM. TEHT in oral developmental toxicity studies at doses up to 1050 mg/kg/day on gestation days 6-19 had no significant toxicological effect, i.e. no effect on sexual maturation or development of the reproductive tract in either male or female offspring (Cosmetic Information Review, 2015).

Microarray technology has been used to demonstrate that the triester TOTM does not repress any of the target genes involved in TMD in the rat male fetal reproductive system, i.e. those genes involved in testosterone synthesis and cryptorchidism (absence of one or both testes from the scrotum) (Elcombe, 2017). This technology, developed by CXR Biosciences® (CXR Biosciences®), used mechanistic understanding of the TMD pathways to measure gene regulation by performing a transcriptomic screen using RNA extracted from fetal rat testis. Unlike DEHP in this same test system, TOTM did not repress any of the TMD target genes extracted from fetal rat testis, indicating that the substance is unlikely to cause testicular dysgenesis in rats as is seen with some phthalate esters.

TABLE 3. SUMMARY OF COMPARISON OF DIESTERS VS. TRIESTERS OF BENZENETRICARBOXYLIC ACID

PARAMETER	DIESTERS	TRIESTERS
Molecular weight	Lower than triesters DEHP 391 g/mol DINP 419 g/mol DIBP 278 g/mol	Higher than diesters MDOT >600 g/mol
Lipophilicity	Very high DEHP log Pow 7.6	Very high MDOT log Pow >4; actual determination not possible due to hydrophobic structure
Water solubility	Slightly soluble DEHP 0.27 mg/L at 25°C	Generally insoluble (Cosmetic Information Review, 2015)
Hydrolysis of ester	Rapid	Slow
Gastrointestinal absorption	Well absorbed (Kluwe, 1982). In rat: At lower doses, most absorbed as hydrolysis products MEHP and 2-ethylhexanol; at higher doses, some unhydrolyzed DEHP is also absorbed. MEHP is well absorbed from the intestine.	Slow Ca. 75% of TEHT excreted unchanged in feces.
Dermal absorption	Minimal	Minimal

Inhalation exposure potential	Not a significant route of exposure	No potential for exposure to vapor
Excretion	Mainly in urine	Mainly in feces
Systemic toxicity		Low: • Rat LD50 for TTDT >5000 mg/kg • Rat LD50 for TEHT = >2000 mg/kg (OECD HPV, 2002) • Rat LC0 for TEHT = >2600 mg/m3 (OECD HPV, 2002) • Rabbit LD0 dermal for TEHT = >2 ml/kg (OECD HPV, 2002)
Effects on fertility and reproductive performance in rats	DEHP: Decreased fertility in rats and mice. DEHP: Negative effects on female reproductive process in rats.	None
Effects on male reproductive system in rats	DEHP: Loss or spermatogenesis in rats and mice, other signs of testicular toxicity following oral exposure (U.S. DHHS, 2002).*** DEHP: Abnormal development of male reproductive tract following perinatal exposure (U.S. DHHS, 2002).	TEHT: Decreased spermatocytes and spermatids in OECD 421* TOTM: No effect on spermatocytes and spermatids in OECD 408 TEHT: No effect on sexual maturation or development of the reproductive tract in either male or female offspring in OECD 414** TOTM: No repression of genes in the TMD pathway indicating that the substance is unlikely to cause testicular dysgenesis in rats as is seen with some phthalate esters.

* Decrease of spermatocytes and spermatids in testis seen in reproductive screening test (OECD TG 421) in P0 animals exposed to 300 and 1000 mg/kg/day TEHT was not seen in subchronic 90-day study (exposure twice as long) at 1000 mg/kg/day of TEHT analog TOTM. Further, treatment at all doses had no effect on reproductive ability or weight of reproductive organs in males or females, had no effect on histopathology of ovaries, and had no effect on the offspring.

** Minor transient effect following in utero exposure (slight, but statistically significant, increase in the number of male animals with retained areolar regions on evaluation at post-natal Day 13) at 1050 mg/kg/day; effect was no longer evident on reexamination on day 18.

*** Multiple studies in rodents have shown negative (adverse) effects on the development, structure and function of the male reproductive tract in rodents (U.S. DHHS, 2002)

CONCLUSION

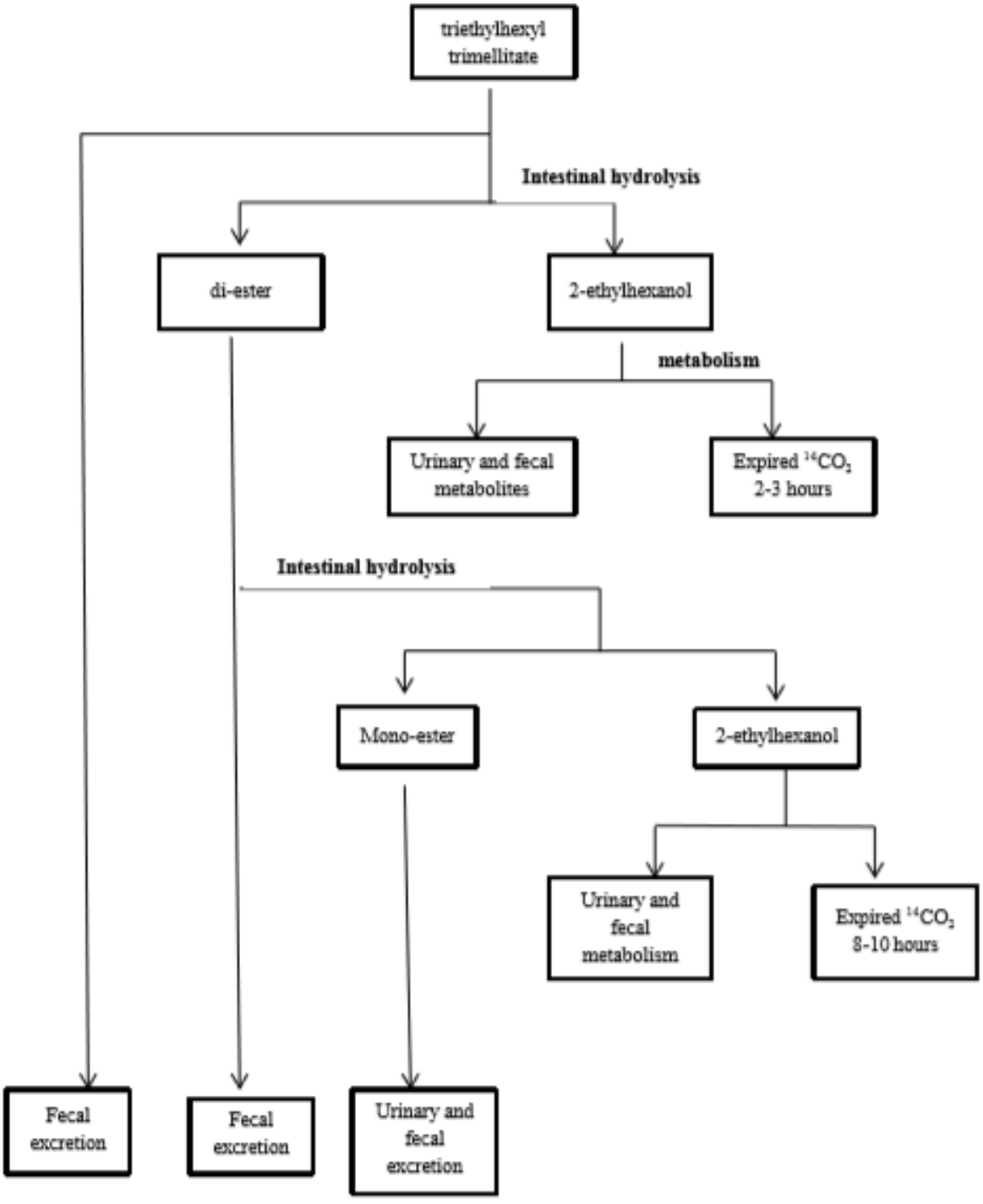
Based upon read-across with related triesters of trimellitic acid, the PMN substance MDOT is expected to exhibit low bioavailability. Based upon studies with DEHP in the rat, the diesters of carboxylic acid are expected to be excreted mainly in the urine as end-products of metabolism, whereas the triesters are expected to be excreted mainly in the feces unchanged. Dermal exposure to the trimellitates is expected to be quite low, and presumably below 1%, whereas exposure through inhalation is expected to be negligible given the low vapor pressure of these chemicals.

Phthalates have been shown to have testosterone-reducing effects in rats due to effects on steroid synthesis following reduction in mRNA coding for target genes involved in testosterone synthesis. Whereas the diester DEHP repressed TMD target genes in fetal rat testis, the triester TOTM, a close analog to the PMN substance, did not repress any of the TMD target genes, indicating that the substance is unlikely to cause testicular dysgenesis in rats as is seen with some phthalate esters including DEHP.

No pathological changes in spermatogenesis or estrus cycle irregularities were seen in male and female reproductive systems following subchronic feeding with the PMN substance MDOT. Microscopic and macroscopic observations of reproductive organs did not indicate any adverse effects on the reproductive organs. Unlike the phthalates, studies with various trimellitates and with trimellitic acid itself have shown no effects on fertility or reproductive function in exposed rats or in their offspring.

Based upon significant differences in chemical structure, physical-chemical properties, toxicokinetic properties, and biological activity, diesters of carboxylic acid are not appropriate read-across substances for triesters of trimellitic acid.

APPENDIX 1


triethylhexyl trimellitate
Intestinal hydrolysis
di-ester
2-ethylhexanol
metabolism
Urinary and fecal metabolites
Expired $^{14}CO_2$ 2-3 hours
Intestinal hydrolysis
Mono-ester
2-ethylhexanol
Urinary and fecal metabolism
Expired $^{14}CO_2$ 8-10 hours
Fecal excretion
Fecal excretion
Urinary and fecal excretion

APPENDIX 2

Summary of existing subacute and subchronic studies on MDOT and similar chemicals:

MDOT was studied in a subchronic (90-day) oral study (OECD Test Guideline 408) (https://echa.europa.eu/registration-dossier/-/registered-dossier/14824/7/6/2)

> Male and female rats were exposed to MDOT at doses up to 500 mg/kg/day by gavage in corn oil. Morphological examination of the seminiferous epithelium (with staging of the spermatogenic cycle, and assessment of integrity of various cell types within the different stages of development) was performed on control and high dose animals. No significant exposure-related changes were seen in the testes of treated animals. No other changes indicative of adverse effects on the reproductive system were seen at any dose (50, 200 and 500 mg/kg/day); in addition to the spermatogenic cycle evaluation, these include oestrus cycle, weights of ovaries and testes, microscopic and macroscopic observations of reproductive organs.
>
> Minimal to mild, and reversible, effects were seen in the livers of treated animals (200 and 500 mg/kg/day); these changes were not considered to be adverse. No pathological changes in spermatogenesis or estrus cycle irregularities were seen in evaluation of the male and female reproductive systems.

MODT was also studied in a Repeated Dose 28-Day Oral Toxicity in Rodents (OECD Test Guideline 407) (https://echa.europa.eu/registration-dossier/-/registered-dossier/14824/7/6/2/?documentUUID=b250fb31-6989-495a-b363-99e6f374a1a2)

> Change in the high dose animals (1000 mg/kg/d) were mild and completely reversible.

TEHT was studied in a subchronic (90-day) oral study (OECD Test Guideline 408) (https://echa.europa.eu/registration-dossier/-/registered-dossier/14933/7/6/2).

> Male and female rats were exposed to TEHT at doses up to 1000 mg/kg/day in the diet. Morphological evaluation of the seminiferous tubule epithelium in the control and high dose animals was conducted in order to evaluate effects of exposure on staging of the spermatogenic cycle. Treatment-related effects that would have been identified in this process include missing germ cell layers or types, retained spermatids, multinucleated or apoptotic germ cells and sloughing of spermatogenic cells into the lumen. In addition, "seminiferous tubules were evaluated with respect to their stage in the spermatogenic cycle and to the integrity of the various cell types within the different stages. Regular layering in the germinal epithelium was noted and the cycle of spermatogenesis was regarded as normal with no treatment-related effect apparent."

Summary of existing reproductive/developmental studies on MDOT and similar chemicals:

MDOT has been studied in a Prenatal Developmental Toxicity Study (OECD Test Guideline 414) (https://echa.europa.eu/registration-dossier/-/registered-dossier/14824/7/9/3).

> Pregnant female rats were exposed to MDOT by gavage (100, 300 and 1000 mg/kg/day) on gd 6-15. Marked maternal toxicity (reduced food consumption and body weight gain, gravid uterus and absolute weight gain) and associated fetal toxicity (reduction in litter weight, fetal weight, and delayed ossification) were seen at the highest dose only. Visceral malformations in mid- and high-dose groups were considered common findings in fetuses and were not considered

treatment-related, while skeletal findings were considered a consequence of low fetal weight and maternal toxicity, rather than an effect of MDOT on the fetus. Incidence of malformations was low and not dose-related (Health Canada review).

TOTM has been studied in a Prenatal Developmental Toxicity Study (OECD Test Guideline 414) (REACH dossier).

> Pregnant female rats were treated with TOTM by oral gavage (0, 100, 300, and 1000 mg/kg/day) on gd 6-19. Maternal animals experienced significantly reduced food consumption, reduced body weight and body weight gain at the highest dose (1000 mg/kg/day). Thus the NOEL = 300 mg/kg/day. The NOAEL for embryo-fetal effects was 1000 mg/kg/day as there were no exposure-related effects on the offspring following exposure during organogenesis in this study.

TEHT was also studies in a Prenatal Developmental Toxicity Study (OECD Test Guideline 414) (https://echa.europa.eu/registration-dossier/-/registered-dossier/14933/7/9/3)

> Two groups of pregnant female rats were treated with TEHT by oral gavage (doses up to 1050 mg/kg/day) on gd 6-19 (prenatal development) or gd 6 to postpartum day 20 (post-natal development). Post-natal examinations showed a slight, but statistically significant, increase in the number of male animals with retained areolar regions on evaluation at post-natal Day 13 at 1050 mg/kg/day; these areolae were no longer evident on reexamination on day 18. (The finding was deemed of questionable toxicological significance. No other treatment related effects were seen in either group. With respect to reproductive parameters, there was no effect on sexual maturation or development of the reproductive tract in either male or female offspring.

TOTM has been studied in a Combined Repeated Dose Toxicity Study with a Reproduction / Developmental Toxicity Screening Test (OECD Test Guideline 422) (Safety Assessment of Trialkyl Trimellitates as Used in Cosmetics, cited REACH dossier)

> Male and female rates were treated with TOTM by oral gavage (0 , 30, 125 and 500 mg/kg/day). [Doses were selected based on results of a 14-day preliminary study in which 1000 mg/kg was shown to cause body weight change, increased liver weight, and edema of the gastric mucosa.] Males were treated for 14 days prior to pairing and for 42 days following pairing. Females were treated from 14 days prior to pairing until day 4 of lactation, at which time the F1 offspring were sacrificed. LOEL = 500 mg/kg.
>
> In the parental generation (P0), the only signs of general toxicity were an increase in liver weight in females and hepatocellular hypertrophy in males, along with a reduction in weight gain in females at gd 7-14 in the highest dose. One female died on gd 23. There was no effect on reproductive function at any of the doses in either sex.
>
> There were no effects on monitored indices in the F1 general, i.e. no effect on pup weight, sex ration, survival and viability indices. NOEL for reproductive/developmental toxicity = 500 mg/kg.

TEHT (ECHA abstract erroneously refers to this as TOTM in several places, but in the OECD SIDS assessment on 3319-31-1, there was clearly an OECD TG 421 run on this chemical, and the test substance is identified as TEHT in the study details) was also studied in a Reproductive / Developmental Toxicity Screening Test) (OECD Test Guideline 421). (https://echa.europa.eu/registration-dossier/-/registered-dossier/14933/7/9/2)

This from ECHA dossier: Rats were exposure to TEHT at doses up to 1000 mg/kg/day by gavage in males (from 14 days before pairing and for 46 days) and females (from 14 days before pairing until day 3 of lactation). {REACH summary (ECHA dossier refers to chemical as TOTM, but it's identified in the details on the test material as TEHT). A slight reduction in the number of spermatocytes and spermatids in the testes was noted in 2/12 and 11/12 males given TEHT at 300 and 1000 mg/kg/day, and a moderate decrease was seen in 1 of the high dose animals. Treatment at all doses had no effect on reproductive ability or weight of reproductive organs in males or females, had no effect on histopathology of ovaries, and no effect on the offspring. "In addition the number of cells/number of spermatids in seminiferous tubules was reduced in males given 300 mg/kg/day TOTM in stages I-VI. In males given 1000 mg/kg/day in stage I-IV numbers of spermatocytes and spermatids were reduced. In stages VII-XIV spermatocyte & spermatid numbers continued to be low & the sertoli cell ratio was also reduced."

From OECD SIDS ((OECD HPV, 2002): NOAEL was considered to be 100 mg/kg bw/day based on testicular toxicity (a decrease of spermatocytes and spermatids in testis) in the 300 and 1000, but not in the 100 mg/kg group.

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