o,p-TSA
HPV TEST PLAN

Submitted to the U.S. Environmental Protection Agency

By

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SUMMARY

Day-Glo Color Corporation has sponsored benzenesulfonamide, ar-methyl-, (CAS# 1333-07-9), also known as o,p-TSA, in the U.S. EPA High Production Volume (HPV) program. Experimental and modeling data exist on several SIDS endpoints for o,p-TSA.

Robust summaries of available studies on o,p-TSA are included in this submission (see Appendix A). Data on o,p -TSA from the EPISuite computer model were used in the absence of experimental data for physical/chemical characteristics. The ECOSAR computer model was used in the absence of experimental data on ecotoxicity endpoints as was data from studies conducted on the ortho and para isomers of TSA. The table below summarizes the endpoints of interest in the HPV program, the available data, and indicates proposed testing.

Endpoint	Sufficient Data Available	Testing Proposed
Physical/Chemical Characteristics	Yes	No
Photodegradation	Yes	No
Hydrolysis	Yes	No
Biodegradation	Yes	No
Transport	Yes	No
Acute Fish Toxicity	Yes	No
Acute Daphnia Toxicity	Yes	No
Acute Alga Inhibition	Yes	No
Acute Toxicity	Yes	No
Genetic Toxicity	Yes	No
Repeated Dose	Yes	No
Reproductive Toxicity	Yes	No
Developmental Toxicity	Yes	No

1.0 INTRODUCTION

Day-Glo Color Corp. has sponsored benzenesulfonamide, ar-methyl- (CAS# 1333-07-9), also known as o,p-TSA (indicating a mixture of ortho and para isomers), in the U.S. HPV program to assess its potential health and environmental hazards, including selected physical/chemical characteristics. Additional synonyms include Ketjenflex® 9 and Santicizer® 9. Data, to support various HPV endpoints, were taken from studies conducted on Ketjenflex® 9 and Santicizer® 9. Typical percent ratios of ortho/para isomers at the time these tests were performed were 30/70 (o/p) for Santicizer® 9 and 40/10/50 (o/m/p) for Ketjenflex® 9. The percent ratios of ortho/para isomers for current commercial products are 20/80 or 40/60. The slight difference in the ratios of isomers between former and current commercial products is not expected to affect the toxicity based on the data for the mixed and individual isomers presented in this test plan.

In the absence of measured environmental and ecotoxicity test data, on o,p-TSA, U.S. EPA validated computer models were used to generate estimated values. In the absence of test data, for health effects endpoints, data from studies conducted on o-TSA (CAS# 88-19-7) and p-TSA (CAS# 70-55-3) isomers were summarized. This is justified since the HPV substance is a combination of these two isomers. The hazard(s) of the HPV substance would not be expected to be greater than the most hazardous endpoint of an individual TSA isomer.

Based on the availability of model and measured data, no testing is proposed for o,p-TSA.

2.0 EVALUATION OF EXISTING DATA AND PROPOSED TESTING

The available data on o,p-TSA has been evaluated and summarized. Data on o,p-TSA from the EPISuite computer model (Ref. 1) were used in the absence of experimental values for physical/chemical properties. The ECOSAR computer model (Ref. 2) was used to estimate toxicity in the absence of measured data for algae. Data for mammalian toxicity was available from Axcentive SARL owned studies as well as from submissions made to the U.S. EPA under TSCA section 8(e). Robust summaries of the studies are included in this submission. The Klimisch (Ref. 3) reliability code was used to determine the reliability of the available studies. The Klimisch rating of 1, 2, 3 and 4 signifies valid without restriction, valid with restriction, not reliable and not assignable, respectively. A literature search of online databases included: TOXNET (TOXLINE, HSDB, IRIS, GENETOX, and CCRIS), PubMed, RTECS, European Chemicals Bureau (IUCLID), SIDS, EPA HPV, NTP, NIEHS, IARC, CIS (Aquire, Datalog, Envirofate, and BIODEG), EPA TSCA 8(e), TSCATS and STN. Several literature articles, on the o-TSA and p-TSA isomers, were identified to satisfy/support endpoints.

Physical/Chemical Properties:

Measured values for o,p-TSA are available for the physical/chemical properties provided in Table 2 below.

Table 2. Measured o,p-TSA Physical/Chemical Data

MW	MP °C (Ref. 4)	BP °C (Ref. 5)	VP mmHg (Ref. 6)	Water Sol. g/L (Ref. 7)
171.22	106	215	$< 3x\ 10^{-7}$	5

The EPISuite model was used to estimate the Log Kow. Using the CAS# for o,p-TSA (1333-07-9) a Log Kow of 0.92 was obtained. A Log Kow of 0.85, from an experimental database match, was also provided in the EPISUITE output.

It should be noted that the physical/chemical values and structure in EPISuite were identical for m-TSA (CAS# 1899-94-1) and o,p-TSA. As Table 3 below demonstrates, endpoint values are comparable for m-TSA (CAS# 1899-94-1), o,p-TSA (CAS# 1333-07-9), p-TSA (CAS# 70-55-3) and o-TSA (CAS# 88-19-7).

Table 3. Comparison of Physical/Chemical EPISuite Model Values of TSA Isomers

Endpoint	m-TSA CAS# 1899-94-1	o,p-TSA CAS# 1333-07-9	p-TSA CAS# 70-55-3	o-TSA CAS# 88-19-7
Log Kow	0.92 (0.85 exper. database match)	0.92 (0.85 exper. database match)	0.92 (0.82 exper. database match)	0.92 (0.84 exper. database match)
Water Solubility	7810 mg/l	7810 mg/l	3160 mg/l	1620 mg/l
MP deg C	91.19	91.19	91.19 (138.5 exper. database match)	91.19 (156.3 exper. database match)
BP deg C	307.16	307.16	307.16 (214 exper. database match)	307.16 (214 exper. database match)
VP mm Hg 25 deg C	3.06 E-004	3.06 E-004	9.56E-005	6.06E-005

According to the EPISuite classification, substances with a Log Kow of < 1 are highly water soluble. The Log Kow values, in Table 3, indicate that all isomers or the combination of o- and p- are water soluble (hydrophilic). In fact this is confirmed by the water solubility values (measured and model values). While the measured water solubility for o,p-TSA (Table 2) is somewhat different from model values (Table 3), all values are within the soluble range classification of EPISuite (>1,000-10,000). Therefore, based on the similarity between the Log Kow values for the individual isomers and confirmation of water solubility from the measured o,p-TSA value, it is appropriate to use the Log Kow value cited in the EPISuite output.

Recommendation: No additional chemical/physical property testing is proposed.

Environmental Fate:

Measured photodegradation rates are not available. However the Atmospheric Oxidation Program (AOPWIN), in EPISUITE, was used to estimate the time required for the material to degrade in the atmosphere. AOPWIN estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The OH

half-life, for all isomers including o,p-TSA, was estimated to be 8.7 days (12-hr day; 1.5E6 OH/cm3). The photodegradation half-life of p-TSA and o-TSA were reported to be 132 days and 315 hours, respectively (Ref. 8 and 9). Table 4 summarizes photodegradation rates.

Table 4. Photodegradation of o,p-TSA and Isomers

Endpoint	Test Substance	Result	Reliability	Reference
Atmospheric Oxidation	o,p-TSA	8.7 days	2	1
Photodegradation	p-TSA	132 days	4 (secondary reference)*	8
Photodegradation	o-TSA	315 hours	4 (secondary reference)*	9

*OECD/SIDS document

The EPISuite model indicates that a hydrolysis half-life cannot be estimated for o,p-TSA or any of the individual isomers.

The EPISuite Level III fugacity model was used to estimate the distribution of o,p-TSA. The modeling results indicate that when distributed equally to air, water and soil, o,p-TSA is primarily distributed to water and soil. Table 5 summarizes the environmental distribution of o,p-TSA and isomers.

Table 5. Environmental Distribution of o,p-TSA and Isomers

Transport/Distribution	m-TSA CAS# 1899-94-1	o,p-TSA CAS# 1333-07-9	p-TSA CAS# 70-55-3	o-TSA CAS# 88-19-7
Air	2.7	2.7	2.7	2.69
Water	46.7	46.7	46.9	46.8
Soil	50.5	50.5	50.3	50.4
Sediment	0.096	0.096	0.096	0.096

o,p-TSA was degraded very slowly in the Shake Flask CO2 Evolution Test (Ref. 10). This test is similar to OECD 301B Modified Sturm Test. However, there are several deviations from the current guideline. o,p-TSA biodegraded 92.9% in a SCAS Test (similar to OECD 302A) (Ref. 11). p-TSA was classified as readily biodegradable in the Closed Bottle Test (OECD 301D) (Ref. 12). p-TSA and o-TSA were not readily biodegradable in MITI (I) (OECD 301C) (Ref. 8 and 9). p-TSA biodegraded 106% by day 28 in the (Repetitive Die Away (RDA) test while o-TSA degraded 27% (Ref. 13). Table 6 summarizes biodegradation of o,p-TSA and isomers.

Table 6. Biodegradation of o,p-TSA and Isomers

Study	Test Substance	Result	Reliability	Reference
SCAS	o,p-TSA	Inherently Biodegradable	2	11
Modified Sturm	o,p-TSA	Not Readily Biodegradable	4 (protocol deviations)	10
Closed Bottle Test	p-TSA	Readily Biodegradable	2	12
RDA	p-TSA	Readily Biodegradable	4 (English translation not available)	13
MITI	p-TSA	Not Readily Biodegradable	4 (secondary reference)*	8
RDA	o-TSA	Not Readily Biodegradable	4 (English translation not available)	13
MITI	o-TSA	Not Readily Biodegradable	4 (secondary reference)*	9

*OECD/SIDS document

Based on the SCAS test, o,p-TSA is considered inherently biodegradable.

Recommendation: No additional environmental fate testing is proposed.

Aquatic Toxicity:

Aquatic studies indicate that o,p-TSA has a low order of acute toxicity to fish and Daphnia as does model data for algae. The ECOSAR model estimated a low order of toxicity, for o,p-TSA to algae using an estimated Log Kow of 0.92. Measured data from algae studies, briefly summarized in the OECD SIDS documents on p-TSA and o-TSA, indicate moderate toxicity to algae. Table 7 below contains available acute aquatic studies on o,p-TSA with the Klimisch reliability code.

Table 7. Ecotoxicity Data of o,p-TSA and Isomers

STUDY	TEST SUBSTANCE	RESULT	RELIABILITY	REFERENCE
96-hr LC50 (Trout)	Santicizer® 9 (o,p-TSA)	120 mg/l	2	14
	p-TSA	100 mg/l	2	15
96-hr LC50 (Bluegill)	Santicizer® 9 (o,p-TSA)	260 mg/l	2	16
	p-TSA	370 mg/l	2	17
96-hr LC50 (Killifish)	p-TSA	435 mg/l	4 (secondary reference)**	8
96-hr LC50 (Killifish)	o-TSA	> 100 mg/l	4 (secondary reference)**	9
48-hr EC50 (Daphnia)	Santicizer® 9 (o,p-TSA)	>1000 mg/l	2	18
24-hr EC50 (Daphnia)	p-TSA	150 mg/l	4 (secondary reference)**	8
48-hr EC50 (Daphnia)	o-TSA	210 mg/l	4 (secondary reference)**	9
21-day LOEL (Daphnia)	p-TSA	150 mg/l	4 (secondary reference)**	8
21-day EC50 (Daphnia)	o-TSA	79 mg/l	4 (secondary reference)**	9
96-hr EC50 (Algae)*	o,p-TSA	768 mg/l	2	2
Algae ChV*	o,p-TSA	41.140 mg/l	2	2
144-hr EC50 (Chlorella pyrenoidosa	p-TSA	80 mg/l	4 (summary only)	19
72-hr EC50 (S. capricornutum)	p-TSA	23 mg/l	4 (secondary reference)**	8
72-hr EC50 S. capricornutum)	o-TSA	57 mg/l	4 (secondary reference)**	9

*ECOSAR
**OECD/SIDS document

Although there is no measured data, the toxicity of o,p-TSA to algae would not be expected to be greater then the toxicity of the individual isomers. Therefore o,p-TSA would, at the most, be expected to be moderately toxic to algae, the most sensitive species, based on studies conducted on the individual isomers.

Recommendation: No additional aquatic testing proposed.

Acute Mammalian Toxicity:

Acute mammalian toxicity studies are available on o,p-TSA. Valid studies indicate a low order of acute toxicity by the oral route and slight irritation to skin and eyes. An acute study also demonstrates a low order toxicity by the dermal route. The report lacks sufficient details. However, based on the lack of toxicity at such a high dose, further testing would not be

substantially additive to the database. Table 8 below contains available acute studies on o,p-TSA with the Klimisch reliability code.

Table 8. Acute Mammalian Toxicity Studies on o,p-TSA

STUDY	TEST SUBSTANCE	RESULT	RELIABILITY	REFERENCE
Acute Oral LD50 (rat)	Ketjenflex® 9	2400 mg/kg	1	20
Acute Oral LD50 (rat)	Ketjenflex® 9	1790 mg/kg	4 (inadequate details)	21
Acute Oral LD50 (rat)	Santicizer® 9	3800 mg/kg	4 (inadequate details)	22
Acute Dermal LD50 (rabbit)	Santicizer® 9	> 7.5 g/kg	4 (inadequate details)	23
Skin and Eye Irritation	Ketjenflex® 9	Slightly Irritating	2	24

Acute studies are also available on the ortho and para isomers. The acute oral LD50 (rat) for o-TSA and p-TSA was greater than 2000 mg/kg in several studies (Refs. 25, 26, 27, and 8). In one study, the LD50 of o-TSA in female rats was reported to be between 1000 and 2000 mg/kg (Ref. 9). The acute dermal LD50 (rabbits), of o-TSA, was reported to be > 7940 mg/kg (Ref. 27). In irritation studies, o-TSA and p-TSA caused slight irritation to skin and eyes (Refs. 24 and 23).

Recommendation: No additional acute mammalian toxicity testing proposed.

Repeated Dose

There are no valid repeat dose studies available on the o,p-TSA mixture. There are 90-day feeding studies in rats and dogs with Santicizer® 9 (o,p-TSA). No effects on survival, hematology, clinical chemistry, gross or microscopic parameters were reported when rats and dogs were fed up to 3000 ppm. However these studies were generated by a laboratory of questionable reputation and cannot be considered valid as the data cannot be verified (Ref. 28 and 29). Repeat dose studies do exist for o-TSA and p-TSA.

A 90-day dietary study with p-TSA, was submitted to US EPA under TSCA section 8(e). Urinary bladder hyperplasia was noted in two males in the high dose group (738 mg/kg/day) and significant reduction in weight gain (males and females) (Ref. 30). In another study submitted on a combined repeat dose oral gavage study, urinary bladder hyperplasia was reported at dose levels of 120, 300 and 750 mg/kg/day as well as involution of the thymus in females in the 300 and 750 mg/kg/day groups (Ref. 31).

The National Toxicology Program (NTP) had conducted a 2-week study with p-TSA in rats and mice. Animals were administered the test article at 750, 1500, 3000, 10000 or 30000 ppm. No treatment related adverse effects were reported (Ref. 32). A 90-day oral gavage study in rats is currently in progress.

In a combined oral gavage study with o-TSA, hepatic centrilobular hypertrophy was noted in rats administered 100 and 500 mg/kg/day. Enhanced incidences and grade of eosinophilic bodies in the kidneys of males in all dose groups (20, 100 and 500 mg/kg/day) and atrophy of the thymus in females in the 500 mg/kg/day group was also reported (Ref. 33). When fed to rats over their lifetime, o-TSA, did not significantly increase the incidence in bladder tumors over controls (Ref. 34).

Table 9 below contains available repeat dose studies on o- and p-TSA with the Klimisch reliability code.

Table 9. Repeat Dose Toxicity Studies on o/p-TSA Isomers

STUDY	Test Substance	RESULT	RELIABILITY	REFERENCE
90-day dietary (rat)	p-TSA	NOEL = 3000 ppm (214 and 248 mg/kg/day, males and females, respectively) LOEL = 10000 ppm (738 mg/kg urinary bladder hyperplasia males; 795 mg/kg/day reduced body weight gain females)	4 (TSCA 8(e) summary only)	30
Combined Repeat Dose Oral Gavage (rat)	p-TSA	NOEL = <120 mg/kg/day LOEL = 120 mg/kg/day (urinary bladder hyperplasia all groups; involution of thymus in 300 and 705 mg/kg/day females)	2	31
2-week Feeding Study	p-TSA	No treatment related adverse effects were reported up to 30000 ppm	2	32
Combined Repeat Dose Oral Gavage (rat)	o-TSA	NOEL = < 20 mg/kg/day LOEL= 20 mg/kg/day (males); 100 mg/kg/day (females) (increased incidence and severity of eosinophilic body in the male kidneys of all groups; centrilobular hepatocyte hypertrophy in 100 and 500 mg/kg/day; atrophy of female thymus in 500 mg/kg/day)	2	33
Two-Generation Lifetime Feeding (rats)	o-TSA	NOEL = 25 mg/kg/day LOEL = 250 mg/kg/day (reduced growth rate)	4 (Only histopathology examinations were reported)	34

Reproductive Toxicity

There is no reproductive study available on o,p-TSA mixture. However, there are studies available to evaluate reproductive endpoints on p-TSA and o-TSA.

In an OECD Combined Repeat Dose and Reproductive/Developmental rat study with p-TSA, there were no effects on mating performance and fertility. The number of corpora lutea and implantations and implantation rate was comparable between treated animals and control animals. Signs of parental toxicity were present in all dose groups (120, 300 and 750 mg/kg/day). Two females in the high dose group (750 mg/kg/day) exhibited a difficult labor (all pups died in these two litters) and a decrease in lactation index. Viability rate for lactation day one was significantly reduced. There were no significant visceral or skeletal findings.

In an OECD Combined Repeat Dose and Reproductive/Developmental rat study with o-TSA, there was no effect on copulation, ovulation and fertility, or on delivery and lactation at doses up to and including 500 mg/kg/day. Signs of parental toxicity were present in the mid (100 mg/kg/day) and high (500 mg/kg/day) dose groups. Males exhibited an increased incidence and severity of eosinophilic bodies in the kidneys in all groups (20, 100 and 500 mg/kg/day). The number of live pups, from this group, on days 0 and 4 of lactation and the birth index tended to be low but not statistically significant. Weights of the pups, were significantly reduced on lactation days 0 and 4.

In a submission to US EPA under TSCA section 8(e) on a two-generation dietary rat study with p-TSA, the NOEL for reproduction and breeding was 10000 ppm Decrease body weight and organ weight changes resulted from exposure to diets containing 3000 and 10000 ppm. F1 pups from the 10000 ppm group exhibited lower body weight and organ weight changes and a delay in vaginal opening and balanopreputial separations. F2 pups exhibited lower body and organ weight changes in the 10000 ppm group. When corrected for mean test article intake 1000 ppm corresponds to 52-78 mg/kg/day and 75-161 mg/kg/day for males and females, respectively, 3000 ppm corresponds to 165-236 mg/kg/day and 232-499 mg/kg/day for males and females, respectively and 10000 ppm corresponds to 566-832 mg/kg/day and 733-1631 mg/kg/day for males and females, respectively.

In a two-generation life time feeding study with o-TSA, there was no effect on reproductive parameters when rats consumed 250 mg/kg/day. Adults exhibited a significant reduction in growth rate at 250 mg/kg/day. There was a statistically significant decrease in litter size on day 1 and 4 postpartum in the 250 mg/kg/day group.

Table 10 below contains available reproductive studies on o- and p-TSA with the Klimisch reliability code.

Table 10. Reproduction Toxicity Studies on o/p-TSA Isomers

STUDY	TEST SUBSTANCE	RESULT	RELIABILITY	REFERENCE
Combined Repeat Dose Oral Gavage (rat)	p-TSA	NOEL (reproduction) = 300 mg/kg/day LOEL (reproduction) = 750 mg/kg/day (delivery dysfunction and suppression of embryos) LOEL (parental toxicity) = 120 mg/kg/day NOEL (developmental) = 300 mg/kg/day (decrease litter weight, lactation index, viability rate in 750 mg/kg day)	2	31
Two Generation Dietary (rat)	p-TSA	NOEL (reproduction) = 10000 ppm LOEL (parental toxicity) = 3000 ppm NOEL (developmental) = 3000 ppm LOEL(developmental) = 10000 ppm (delay vaginal opening and balanopreputial separation)	4 (TSCA 8(e) summary)	35
Combined Repeat Dose Oral Gavage (rat)	o-TSA	NOEL (reproduction) = 500 mg/kg/day LOEL (parental toxicity) = 20 mg/kg/day NOEL (developmental) = 100 mg/kg/day; (non-significant reduction in number of live pups and birth index on days 0 and 4 lactation and significantly reduced weights in 500 mg/kg/day group)	2	33
Two-Generation Dietary (rat)	o-TSA	NOEL (reproduction) = 250 mg/kg/day LOEL (parental toxicity) = 250 mg/kg/day NOEL (developmental) = 25 mg/kg/day (decreased litter size at 250 mg/kg/day and lower pup weight)	4 (Details of reproductive parameters such as ovulation, implantation, delivery and lactation analyses were not provided)	34

Developmental Toxicity

There is one valid developmental study available the on o,p-TSA mixture.

Santicizer® 9, a mixture of 32% o-TSA and 68% p-TSA was administered to pregnant rats during gestation days 6-15 at 50, 250 or 500 mg/kg/day. There was a significant reduction in maternal body weight gain in the mid and high dose group during treatment. Weight loss was noted during this interval. The test article was fetotoxic at 250 and 500 mg/kg/day as demonstrated by increased postimplantation loss in the presence of maternal toxicity. There was a significant difference in fetal body weight and an increase in unossification of sternebrae at 500 mg/kg/day but no teratogenic effects (Ref. 36). In the range finder to this study, pregnant rats were administered Santicizer® 9 at 100, 500, 1000, 1500 or 2000 mg/kg/day during gestation. Maternal toxicity was observed at dose levels of 500 mg/kg/day and greater. At 1500 and 2000 mg/kg/day signs of developmental toxicity included increased resorptions and postimplantation loss (Ref. 37).

While this is the only study available on the substance with mixed isomers, additional studies have been conducted on the individual ortho and para isomers (Table 11).

In a submission to US EPA under TSCA section 8(e) on a dietary study, pregnant rabbits were given diets, on gestations 7-29, containing p-TSA.

Reduced body weight and food consumption was noted in dams at dietary levels of 3000 ppm (113 mg/kg/day) and 11000 ppm (367 mg/kg/day). Dams exposed to the 1000 ppm (41 mg/kg/day) dietary level did not exhibit any toxicity. There was no effect on reproductive parameters in any treatment group.

There was a statistically significant increase in the mean litter proportion of fetuses with vertebral anomaly with or without associated rib anomaly in the 11000 ppm group. However, there was a confounding factor of environmental stress to dams, prior to treatment, resulting in a low pregnancy rate and subsequently an insufficient number of pregnant animals. Additional animals were therefore added to each group. Only fetuses from the initial pregnant dam group exhibited the vertebral anomaly. Fetuses from dams added to the study did not display the anomaly. Additionally, this vertebral anomaly was not observed in fetuses, from dams in the range-finding study, exposed to dietary levels up to 20000 ppm. Therefore, the results from this study are equivocal due to the confounding environmental stress (Ref. 38).

A developmental study was conducted to determine the effect of o-TSA on the urinary tract of rats exposed during gestation and postnatally (Ref. 39). The test article was administered to dams by oral gavage beginning day one of pregnancy until the pups were weaned, day 21 postpartum. After weaning the pups were fed diets containing equivalent dose levels of the test article as their mother, 40, 100 or 250 mg/kg/day.

There were no clinical signs of maternal toxicity and no effect on reproductive parameters. Pups, of both sexes, exposed during gestation, lactation and up to 105 days postpartum, exhibited a dose related increase in the incidence of urinary calculi and in the 100 and 250 mg/kg/day dose groups, bladder hyperplasia in 105-day old males.

When the same study was conducted with dietary equivalents, there was a significant dose response for the incidence of renal calculi in 8-day old pups but not older pups. Eight-day old pups in the 250 mg/kg/day group had a significant increase in focal epithelial hyperplasia.

Table 11. Developmental Toxicity Studies on o,p-TSA and Isomers

STUDY	TEST SUBSTANCE	RESULT	RELIABILITY	REFERENCE
Developmental Oral Gavage (rats)	Santicizer® 9 (o,p-TSA)	NOEL (maternal) = 50 mg/kg/day LOEL (maternal) = 250 mg/kg/day NOEL (fetal) = 50 mg/kg/day LOEL (fetal) = 250 mg/kg/day (fetotoxic at 250 and 500 mg/k/day; unossification of sternebrae at 500 mg/kg/say; Not teratogenic)	2	36 and 37
Developmental Dietary (rabbits)	p-TSA	NOEL (maternal) = 1000 ppm (41 mg/kg/day) LOEL (maternal) = 3000 ppm (113 mg/kg/day) NOEL (fetal) = 3000 ppm LOEL (fetal) = 11000 ppm (367 mg/kg/day-vertebral anomalies)	4 (TSCA 8(e) summary and equivocal results due to confounding environmental stress)	38
Developmental Oral Gavage (rats)	o-TSA	NOEL (maternal) = 250 mg/kg/day NOEL (fetal) = 250 mg/kg/day for 8 and 15 day old pups LOEL (fetal) = 40 mg/kg/day for 21 and 105 day old pups (dose related urinary calculi at 40, 100 and 250 mg/kg/day; epithelial hyperplasia in the bladder in the males at 100 and 250 mg/kg/day)	4 (non-guideline study, no maternal toxicity)	39

It should be emphasized that the only study which reported malformations was the 8(e) submission conducted with p-TSA on rabbits. However, the results from this study are equivocal due to confounding factors of environmental stress as noted in the 8(e) letter. No such findings were reported in any of the combined repeat dose reproductive/developmental studies or in the reproductive studies, conducted on o-TSA and p-TSA summarized above.

Recommendation: No additional testing proposed for repeated dose/reproductive/developmental toxicity.

Mutagencity/Genotoxicity:

Two valid *in vitro* microbial assays were conducted with o,p-TSA products. Both were negative, with and without metabolic activation, in Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100 and Saccharomyces D4 (Ref 40 and 41).

In a study conducted by the Japanese Ministry of Health and Welfare, p-TSA was not mutagenic to Salmonella typhimurium or Escherichia coli with and without metabolic activation when tested up to 5000 ug/plate (Ref. 8). In a National Toxicology Program (NTP) Salmonella/E. coli mutagenicity test, p-TSA was not mutagenic when tested up to 10000 ug/plate (Ref. 42)

The OECD/SIDS document, for o-TSA, cites seven bacterial *in vitro* mutation assays. Six were negative and one was weakly positive. In the weakly positive study there was a non-dose response 2-3 fold increase in TA98 with activation. The weakly positive result could not be confirmed when the study was duplicated (Ref. 9).

p-TSA was negative in the Mouse Lymphoma Forward mutation assay when tested without metabolic activation and positive with activation (high dose only) when tested up to 2000 ug/plate (Ref. 45).

While no chromosomal aberration studies have been conducted on the mixture of o- and p-TSA isomers, mammalian (CHL cells) *in vitro* chromosomal aberrations studies have been conducted on o-TSA and p-TSA with and without metabolic activation (Ref 43 and 44). Both were negative. Therefore, the o,p-TSA mixture is not expected to induce chromosomal aberrations.

o-TSA was also negative in the Ouabain-resistant mutation assay with human RSa Cells and did not induce chromosomal aberrations in CHO-K1 cells (Ref. 9).

p-TSA was negative in three micronucleus assays (Ref. 42 and 46, 47).

o-TSA, administered orally and by i.p. injection to mice, did not induce micronuclei in bone-marrow cells (Ref. 47).

o-TSA did not induce morphological transformation in BHK 21/Cl 13 cells (Ref. 47)

Both o-TSA and p-TSA were considered weakly positive in a sex-linked recessive lethal assay (SLR) in Drosophila melanogaster. However other SLR assays conducted on the isomers were negative (Refs. 8 and 9).

Table 12 summarizes mutagenicity/genotoxicity studies on o,p-TSA and isomers.

Based on a weight of evidence approach, o,p-TSA is not expected to be mutagenic or genotoxic.

Recommendation: No additional genotoxicity testing proposed.

Table 12. Mutagenicity/Genotoxicity Studies on o,p-TSA and Isomers

Test	Test Substance	Result	Reliability	Reference
Bacterial Reverse Mutation	o,p-TSA	Negative	2	40 and 41
Bacterial Reverse Mutation	p-TSA	Negative	4 (secondary reference)*	8
Bacterial Reverse Mutation	p-TSA	Negative	2	42
Bacterial Reverse Mutation	o-TSA	Six Negative/ One Weak Positive	4 (secondary reference)*	9
Mouse Lymphoma	p-TSA	Negative without activation/ Positive with activation	1	45
Chromosomal Aberrations	p-TSA	Negative	2	43
Chromosomal Aberrations	o-TSA	Negative	2	44
Micronucleus	p-TSA	Negative	2 and 1	42 and 46
Micronucleus	p-TSA	Negative	4 (secondary reference)**	47
Micronucleus	o-TSA	Negative	4 (secondary reference)**	47
Morphological transformation	o-TSA	Negative	4 (secondary reference)**	47
Ouabain Resistance	o-TSA	Negative	4 (secondary reference)*	9
SLR	p-TSA	Negative/Weak Positive	4 (secondary reference)*	8
SLR	o-TSA	Negative/Weak Positive	4 (secondary reference)*	9

*OECD/SIDS document
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Other Studies

In addition to the above studies, the OECD/SIDS document summaries several carcinogenicity studies and concludes that o-TSA is not carcinogenic (Ref. 9).

Therefore, based on a weight of evidence approach from repeat dose and lifetime studies, o,p-TSA is not expected to be carcinogenic.

3.0 USE AND EXPOSURE INFORMATION

o,p-TSA is used as an intermediate in the production of pigments and is totally consumed in the production of the end product. Therefore, both occupational and environmental exposure are expected to be minimal.

o,p-TSA environmental exposure and fate as well as occupational exposure are summarized in Appendix B of this submission.

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