AR201-14074

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18th November 2002

Administrator
US Environmental Protection Agency
PO Box 1473
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Attention: Chemical Right-to-Know Program

HPV TEST PLANS AND ROBUST STUDY SUMMARIES FOR 2-CYCLOHEXENE-1-OCTANOIC ACID, 5 (OR 6)-CARBOXY-4-HEXYL (CAS No 53980-88-4)

Dear Ms Whitman

On behalf of MeadWestvaco Corporation, I am pleased to submit the Test Plans and Robust Study Summaries for 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl under their commitment to the US High Production Volume (HPV) Challenge Program.

The submission includes an electronic copy of the test plans / robust study summaries and of this letter in pdf format, and a paper copy of the test plan / robust study summary. The sponsor identification number for MeadWestvaco Corporation is

I understand that this information will be posted on the internet for a 120-day comment period after initial review by the EPA, and that MeadWestvaco Corporation will have an opportunity to respond to all comments generated by or provided to EPA.

Should you have any questions concerning this submission please contact either me or the sponsor of this chemical, MeadWestvaco Corporation, Chemical Division, 3950 Faber Place Drive, North Charleston, South Carolina 29405, Attention Frank, L Lambert, Environmental and Technical Director.

Yours sincerely

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AR201-14074A

HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

TEST PLAN

for

2-CYCLOHEXENE-1-OCTANOIC ACID, 5 (OR 6)-CARBOXY-4-HEXYL

CAS NO 53980-88-4

Submitted to the US EPA

by

MeadWestvaco Corporation

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Test Plan for 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl

Test Plan for 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl

Summary

MeadWestvaco Corporation is sponsoring 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl under the HPV Chemical Challenge Program. This test plan addresses the available data on this substance and proposes testing as appropriate.

This sponsored substance is a branched, C-21 dicarboxylic acid composed of saturated alkyl chains and a cyclohexene branch. The substance is supplied commercially as Westvaco DIACID® 1550. This is a class 2 product containing several components, being a mixture of about 60-70% of the C-21 diacid, 20-25% unreacted C-18 monoacid and 5-10% C-36 dimer acid.

In general, testing has and will be carried out using Westvaco DIACID® 1550. In some cases it is desirable to maximize the concentration of test substance dissolved in water and so the potassium salt has been used, as the commercial product Westvaco DIACID® H-240.

Westvaco DIACID® 1550 is manufactured by a patented process from tall oil fatty acids, which are obtained by the fractional distillation of crude tall oil, a by-product from the pulping of pine trees. Tall oil fatty acids and some other similar substances have been sponsored under the HPV chemical challenge program by the Pine Chemicals Association HPV task force (PCA), of which MeadWestvaco Corporation is a member.

Although there are some similarities between 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl and the chemicals sponsored by the PCA, there are also differences. Hence, data on tall oil fatty acids and other substances have not been read across to meet SIDS endpoints for 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl. However, these data have been used as supporting information to gain a broader view of the properties of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl in cases where testing has not been performed, and also in assessing requirements for further testing.

Physicochemical properties

The **melting point** will not be determined because the substance is a liquid. Also, as a class 2 substance, it is unlikely to give a sharp freezing point.

The **boiling point** cannot be determined because the substance will decompose, possibly explosively, before it boils.

The **vapor pressure** is negligible under ambient conditions and will not be determined.

Adequate data are available for the **partition coefficient**.

The **water solubility** will be determined.

Environmental Fate

Adequate **biodegradation** data are available.

Hydrolysis will not be determined because the substance has a very low solubility in water and lacks a functional group that would be susceptible to hydrolysis.

Determination of **photodegradation** is not relevant since the vapor pressure of the substance is essentially zero and it will not enter the atmosphere.

Transport and distribution between environmental compartments will not be determined due to the inability to provide usable inputs for the required modelling.

Ecotoxicity

The potassium salt has been used in tests to determine the **acute toxicity to fish, daphnia and algae**. Adequate data are available and no further testing is proposed.

Human health Effects (Mammalian) Toxicity

Adequate data is available for **bacterial and non-bacterial genotoxicity** and **acute oral toxicity**. No data are available for **subchronic toxicity**, **toxicity to reproduction** and **developmental toxicity.** Testing following the OECD 422 combined test guideline is proposed to address these SIDS endpoints.

I Description of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl

A 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl

The compound 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl (CAS Registry Number 53980-88-4) is being sponsored by MeadWestvaco Corporation under the US EPA HPV chemical challenge program. This substance is a branched, C-21 dicarboxylic acid composed of saturated alkyl chains and a cyclohexene branch. The substance is supplied commercially as Westvaco DIACID® 1550. This is a class 2 product containing several components, being a mixture of about 60-70% of the C-21 diacid, 20-25% unreacted C-18 monoacid and 5-10% C-36 dimer acid.

Westvaco DIACID® 1550 is manufactured by a patented process from tall oil fatty acids, which are obtained by the fractional distillation of crude tall oil, a by-product from the pulping of pine trees. Westvaco DIACID® 1550 is used as a surfactant in a wide range of applications. It also provides lubrication and corrosion protection in metalworking applications and acts as an hydrotrope for nonionic surfactants in alkaline cleaner formulations.

B Related Compounds

There are several related materials that should be considered, as data on the properties of these substances may potentially be read across to meet data requirements for 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl. These related substances have been sponsored under the HPV chemical challenge program by the Pine Chemicals Association HPV task force (PCA), of which MeadWestvaco Corporation is a member.

The first set of related compounds are six substances forming the “Tall Oil Fatty Acids and Related Substances” group. Tall oil fatty acids (CAS Registry Number 61790-12-3) are of obvious relevance since they are the precursor of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl and are present in the Westvaco DIACID® 1550 product. Tall oil fatty acids are also the dominant member of the “Tall Oil Fatty Acids and Related Substances” group, being the substance with by far the greatest production volume of the substances in this group. Tall oil fatty acids typically contain a combined total of about 80% of oleic and linoleic acids. These are unsubstituted, linear, C-18, mono- or di- unsaturated monocarboxylic acids.

Also of some relevance in the “Tall Oil Fatty Acids and Related Substances” group are fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear (CAS Registry Number 68955-98-6), also known as monomer acid. This substance is a complex mixture. It typically contains about 36% oleic and elaidic acids, which are *cis* and *trans* forms of unsubstituted, linear C-18 monounsaturated monocarboxylic acids. Fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear also typically contain 28% of other branched C-18 acids and 24% of other C-18 acids, probably cyclic acids of unknown structure. Fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear have

similarities to tall oil fatty acids but contain a much lower level of unsaturation and also contain some branched and cyclic chains. Because of this, fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear share some characteristics with 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl. There are however differences in the numbers of carbon atoms and carboxylic acid groups in the molecules.

The proposed test plan for the "Tall Oil Fatty Acids and Related Substances" group was posted on the HPV web site on 14th June 2001. The EPA provided comments on this test plan on 3rd December 2001 and the PCA responded to all comments with a revised test plan on 5th March 2002.

This revised test plan for the "Tall Oil Fatty Acids and Related Substances" group will provide data on the water solubility, partition coefficient and biodegradability of all six members. However, toxicological testing will only be carried out on tall oil fatty acids and fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear. The toxicological properties of the other group members will be read across from these substances.

The other group of chemicals sponsored by the PCA that is of interest is the "Fatty Acid Dimers and Trimer" group. The dimer is manufactured from C-18 unsaturated fatty acids and consists of many geometric isomers of C-36 dicarboxylic acids with a low level of unsaturation. The compounds may be acyclic, cyclic, aromatic or polycyclic. Apart from the presence of C-36 dimer in Westvaco DIACID® 1550, the relevance of dimer is that it is also composed of branched dicarboxylic acids, although they have a much higher molecular weight than 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl and a wide range of isomeric structures.

The proposed test plan for this category was posted on the HPV web site on 4th April 2002. The EPA replied on 30th July 2002 but a revised test plan has, at the time of writing, not been posted on the EPA HPV web site. Dimer is the primary substance in this group, which has three other members. It is proposed that water solubility, partition coefficient and biodegradation data will be developed for these other three members but that toxicological data will be read across from that for dimer.

C Use of Data on Related Compounds

Taken together, these related substances (tall oil fatty acids, dimer and fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear) share some characteristics with 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl. They are all long chain alkyl carboxylic acids with various degrees of branching and unsaturation. The major feature that distinguishes 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl from all the related compounds is that it is a C-21 compound. Also, it is a diacid, a feature shared only with the dimer molecule.

Hence any proposed read across of data from these other substances to 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl needs to be supported by evidence of comparability. This additional information must show that other, relevant, properties of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl are similar to those of the compounds from which data are being read across. This is an important consideration

since the PCA, following comments from the EPA, decided not to read across data from tall oil fatty acids to fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear, even although these substances are within a single chemical category.

In view of these concerns regarding the read across of data and the differences and similarities between the molecules, it is proposed that there is no direct read across of data from these related substances to 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl. However, the proposed test plan for to 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl bears many similarities to the proposed and revised test plans for the related substances, reflecting the common features of all four substances.

D Choice of Test Substance

The sponsored chemical, 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl is not supplied and used as an isolated molecule but as the major constituent of a commercial product, Westvaco DIACID® 1550. Therefore, testing, in general, will be carried out using Westvaco DIACID® 1550 as the test substance as this is the substance used commercially.

However, 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl and Westvaco DIACID® 1550 have only low solubilities in water. For the evaluation of some properties, the test substance used was Westvaco DIACID® H-240. This is a 40% solution of the potassium salt of Westvaco DIACID® 1550 and maximizes the solubility of the test substance. A similar approach to provide greater solubility of the test substance has been adopted by the PCA in the revised test plan for the “Tall Oil Fatty Acids and Related Substances” group, with fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear being tested as the sodium salt.

II Review of Existing data and Development of Test Plan

A Overview

The data available on the SIDS endpoints of concern for 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl have been subject to a comprehensive review. Both the current state of knowledge and the reliability of the data have been assessed, together with corresponding data and test plans for the related compounds. A test plan has subsequently been drawn up so that all relevant SIDS endpoints may be addressed satisfactorily to meet the commitment of MeadWestvaco Corporation in sponsoring 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl under the EPA HPV Chemical Challenge Program.

The availability of data for 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl (as Westvaco DIACID® 1550) is shown in Table 1. This table also shows data gaps that will be addressed by additional testing and the corresponding plans from the PCA for tall oil

fatty acids, dimer and fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear.

Table 1
Matrix of Available Adequate data and Proposed Testing on 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl and Related Substances.

SIDS endpoint	Westvaco DIACID® 1550 (proposed)	Tall Oil Fatty Acids (revised plan)	Monomer Acid (b) (revised plan)	Dimer (original plan)
melting point	do not test	do not test	do not test	do not test
boiling point	do not test	do not test	do not test	do not test
vapor pressure	do not test	do not test	do not test	do not test
water solubility	test	test	test	test
partition coefficient	available	test	available	test
biodegradation	available (a)	available	test	available
hydrolysis	do not test	do not test	do not test	do not test
photodegradation	do not test	do not test	do not test	do not test
transport / distribution	do not test	do not test	do not test	do not test
toxicity to fish	available (a)	test	test	test
toxicity to *Daphnia*	available (a)	test	test	test
toxicity to algae	available (a)	test	test	test
acute toxicity	available	available	test	available
subchronic toxicity	test (c)	available	test	available
mutagenicity – bacterial	available	available	test	available
mutagenicity – mammalian	available	available	test	available
toxicity to reproduction	test (c)	available	test	available
developmental toxicity	test (c)	available	test	test

(a) – test carried out on potassium salt, Westvaco DIACID® H-240
(b) – "monomer acid" is alternative name for "fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear"
(c) – combined test following OECD guideline 422

As may be seen, the proposed test plan will result in data availability for 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl identical to that of the related substances (tall oil fatty acids, dimer and fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear) in the two test groups sponsored by the PCA.

B Evaluation of Physicochemical Data and Proposed Testing

The basic physicochemical data required in the SIDS battery are melting point, boiling point, vapor pressure, partition coefficient and water solubility.

Class 2 substances are composed of a complex mixture of molecules that are often difficult to characterize. Westvaco DIACID® 1550 is derived from natural sources of variable composition that cannot be represented by a definite chemical structure. Due to this complex composition some measurements of physical properties are likely to produce results that are erroneous, difficult to interpret or meaningless.

In principle, testing of physicochemical properties could be carried out on 2-cyclohexene-1-octanoic acid, 5-carboxy-4-hexyl or 2-cyclohexene-1-octanoic acid, 6-carboxy-4-hexyl, if these compounds were isolated from the Westvaco DIACID® 1550 product. However, such information would be of little practical use in evaluating the properties of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl under the HPV Chemical Challenge Program. Isolation and testing using 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl is therefore not proposed within the test programme.

B1 Melting Point

Westvaco DIACID® 1550 is a liquid under normal conditions and determination of the melting point is not applicable. While consideration can be given to measurement of a freezing point, this is not likely to provide any useful information since Westvaco DIACID® 1550 is a complex mixture. It is therefore likely not to provide a defined freezing point but to display freezing behavior over a range of temperatures. It is therefore proposed that determination of the melting point should not be attempted.

B2 Boiling Point

Westvaco DIACID® 1550 is a non-volatile liquid at ambient temperatures and will decompose if heated to high temperatures. According to the material safety data sheet, the boiling point is in excess of 260ºC, while the flash point is 235ºC. Explosive mixtures may be formed at temperatures at, above, or, in some circumstances, below the flash point. Hence the boiling point will not be determined as such measurements serve no value within the HPV Chemical Challenge Program, while performing the required experiment would be potentially dangerous.

B3 Vapor Pressure

The vapor pressure of Westvaco DIACID® 1550 at ambient temperatures is effectively zero, and experimental measurement is inappropriate.

B4 Water Solubility

The solubility in water of Westvaco DIACID® 1550 will be determined following OECD guideline 105.

B5 Octanol – Water Partition Coefficient

Testing has been recently been performed to generate adequate data showing that the log K_{ow} for Westvaco DIACID® 1550 at pH 2 is approximately 7.09, this being the mean of two peaks observed under the experimental conditions. No further testing to ascertain the

partition coefficient is proposed, although the report notes that the values obtained are outside the specified range (0 to 6) for the method used. Multiple values for the partition coefficient would be expected since the substance is a mixture of several compounds.

Summary of Physicochemical Properties Testing: The water solubility will be determined. Adequate data are available for the partition coefficient. Tests for melting point, boiling point and vapor pressure are inappropriate.

C Evaluation of Environmental data and Proposed Testing

The fate and behavior of a chemical in the environment is determined by the rates of the most important transformation (degradation) processes. The basic environmental fate data covered by the HPV Chemical Challenge Program includes biodegradation, stability in water (hydrolysis as a function of pH), photodegradation and transport and distribution between environmental compartments.

C1 Biodegradation

Data and robust study summaries are available. Because of the low solubility in water of Westvaco DIACID® 1550, the tests were carried out using the potassium salt, Westvaco DIACID® H-240. When tested for ready aerobic biodegradability following OECD guideline 301E the material was found not to be readily biodegradable, showing 63% degradation after 35 days. However, when tested for biodegradation using anaerobic sludge as specified in EPA OTS method 40 CFR 796.3140 (now EPA OPPTS harmonized guideline 835.3400), biodegradation of up to 84% was observed within 56 days. This method is used for screening for the anaerobic biodegradability of organic compounds. A high biodegradability result in this test provides evidence that the test substance will be biodegradable in sewage treatment plant anaerobic digestors and in many natural anaerobic environments such as swamps, flooded soils, and surface water sediments.

The available data are considered adequate and no further testing is proposed.

C2 Hydrolysis

Hydrolysis as a function of pH is a measure of the stability of a substance in water. Westvaco DIACID® 1550 does not contain any organic functional groups susceptible to hydrolysis. In addition, low solubility in water often limits the ability to determine hydrolysis and Westvaco DIACID® 1550 has a very low solubility in water. Therefore, this test material is expected to be stable in water and testing of hydrolytic stability as a function of pH is not considered applicable.

C3 Photodegradation

No information is available on the photodegradation of Westvaco DIACID® 1550. However, since the substance is not volatile, it will not enter the atmosphere and be subject to photodegradation. Additionally, the chemical structures suggest that the

molecules would not be susceptible to breakdown by a photodegradative mechanism. For these reasons, testing for photodegradation is not considered applicable.

C4 Transport and Distribution

Transport and distribution between environmental compartments is intended to determine the ability of a chemical to move and partition in the environment. Such information is generated from models such as the level III model from the Canadian Environment Modelling Centre at Trent University. Use of these models requires the input of a range of parameters. For class 2 substances the required inputs are often not available or impossible to determine. Use of the model would not only require the input of multiple parameters but also potentially present multiple outputs for individual constituents of the product. These would not form a reasonable representation of the environmental distribution of the product. For these reasons, even although no information is available on the environmental transport and distribution of Westvaco DIACID® 1550, no work to assess the environmental transport and distribution of this substance is proposed.

Summary of Environmental Fate Testing: Biodegradation data are available. Determinations of photodegradation, hydrolysis and transport and distribution between environmental compartments are inapplicable.

D Evaluation of Ecotoxicity Data and Proposed Testing

The basic ecotoxicity data that are part of the HPV Chemical Challenge Program are acute toxicity to fish, daphnia and algae. Because of the low solubility in water of Westvaco DIACID® 1550, these tests were carried out using the potassium salt, Westvaco DIACID® H-240. This maximizes the concentration of test material to which the test organisms can be exposed while minimizing the potential for physical toxicity arising from the formation of features such as dispersions or critical micelles.

D1 Acute Toxicity to Fish

Testing has been performed and a robust test summary is available. The 96 hour LC_{50} to the fresh water minnow, *Pimephales promelas*, was found to be 15 mg/l. The No Observed Effect Concentration was 9.8 mg/l. No further testing is proposed.

D2 Acute Toxicity to Daphnia

Testing has been performed and a robust test summary is available. The 48 hour LC_{50} to the water flea, *Daphnia pulex*, was found to be 22.5 mg/l. The No Observed Effect Concentration was 9.8 mg/l. No further testing is proposed.

EPA guidance (*Federal Register,* Vol 65 No 248 page 81695) recommends that, for chemicals with a log K_{ow} value greater than 4.2, a test of chronic toxicity to *Daphnia* is carried out rather than tests of the acute toxicity of the material to *Daphnia* and fish. This is due to concerns about the potential for bioaccumulation of such test materials. However,

the very high partition coefficient and the very low water solubility of Westvaco DIACID® 1550 together indicate that bioaccumulation is unlikely. Since data from acute tests are already available, it is considered that a 21-day test with *Daphnia* would produce no additional data of benefit, although considerable practical difficulties could arise in attempting to perform such a test.

D3 Acute Toxicity to algae

Testing has been performed and a robust test summary is available. The 96 hour EC_{50} to S*elenastrum capricornutum* was found to be 87.6 mg/l. The approximate No Observed Effect Concentration was 32 mg/l. No further testing is proposed.

Summary of Ecotoxicity Testing: The acute toxicity of Westvaco DIACID® H-240 (the potassium salt of DIACID® 1550) to fish, daphnia and algae has been determined. Adequate data are available and no further testing is proposed.

E Evaluation of Human Health Effects and Proposed Testing

The basic toxicity data required under the HPV Chemical Challenge Program are acute and repeated dose toxicity, bacterial and non-bacterial genotoxicity and developmental and reproductive toxicity.

E1 Acute Oral Toxicity

Testing has been performed with Westvaco DIACID® 1550 and a robust test summary is available. The LD_{50} to Sprague-Dawley rats, following a non-standard protocol, was found to be 6176 mg/kg bw. This study has a Klimish Reliability code of 2 – reliable with restrictions.

In other testing reported by the PCA, the acute oral toxicity of dimer, in three tests following the OECD 401 protocol, has been found be >5000 mg/kg bw for Wistar rats and >2000 mg/kg bw for Sprague-Dawley rats. The acute oral toxicity of tall oil fatty acids, also following the OECD 401 protocol, was >10000 mg/kg bw. The revised test plan from the PCA for the "Tall Oil Fatty Acids and Related Substances" group also includes determination of the acute oral toxicity of the sodium salt of fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear.

Although existing data on the acute oral toxicity of Westvaco DIACID® 1550 were generated using a non-standard protocol, all currently available test results indicate that these long chain fatty acids have low acute oral toxicity. Therefore, no further testing of Westvaco DIACID® 1550 is proposed.

E2 Subchronic Toxicity

No information is available on the subchronic toxicity of Westvaco DIACID® 1550. The PCA has reported that tall oil fatty acids have been tested for repeat dose toxicity in a 90

day study and found to have low toxicity, with a no observed effect level (NOEL) of approximately 2500 mg/kg bw/day. Dimer has been similarly tested and also found to be of low toxicity. Although a NOEL was not established, the tests revealed a no observed adverse effect level (NOAEL) of approximately 100 mg/kg bw/day. The revised test plan for the “Tall Oil Fatty Acids and Related Substances” group also includes determination of the subchronic toxicity of the sodium salt of fatty acids, C-16 to C-18 and C-18 unsaturated, branched and linear.

The need to establish the subchronic toxicity of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl has been carefully considered. All available data for acute and subchronic toxicity of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl, tall oil fatty acids and dimer indicate low toxicities. This leads to the expectation that 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl also has a low subchronic toxicity and that that further testing, which would require the use of vertebrate animals, is not justified. On the contrary, subchronic toxicity is a basic data requirement within the HPV Chemical Challenge Program, and the absence of data for this end-point, together with the limited reliability of the available acute toxicity data suggests that testing of Westvaco DIACID® 1550 using OECD method 408 is required. However, for reasons described below in sections E5 and E6, a combined subchronic toxicity and reproductive / developmental toxicity screen test (OECD 422) of Westvaco DIACID® 1550 is proposed.

E3 Bacterial Genotoxicity

Testing has been performed on Westvaco DIACID® 1550 and a robust test summary is available. The results show no evidence of mutagenicity against a range of bacteria in either the presence or absence of metabolic activation when tested at the limits of solubility and cytotoxicity.

The available data are considered adequate and no further testing is proposed.

E4 Non-bacterial Genotoxicity

Testing has been performed on Westvaco DIACID® 1550 and a robust test summary is available. The results show no significant increase in chromosomal aberrations in cultured Chinese hamster ovary cells in either the absence or presence of metabolic activation.

The available data are considered adequate and no further testing is proposed.

E5 Toxicity to Reproduction

No information is available on the toxicity to reproduction of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl. The PCA has reported that tall oil fatty acids have been tested for reproductive toxicity in a procedure consistent with OECD method 415. Tall oil fatty acids have no effect upon reproductive capabilities at doses of approximately 5000 mg/kg bw/day. The PCA has also reported that the test for the subchronic toxicity of dimer included histopathology of the reproductive organs and showed no evidence of toxicity to these organs at any dose level.

It is therefore considered that 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl is unlikely to be toxic to reproduction. However, no data are available. Since other data gaps have been identified, as described in sections E2 and E6, a combined subchronic toxicity and reproductive / developmental toxicity screen test (OECD 422) of Westvaco DIACID® 1550 is recommended.

E6 Developmental Toxicity

No information is available on the developmental toxicity of of 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl. The PCA has reported that tall oil fatty acids have been tested for developmental toxicity in a procedure consistent with OECD method 415. Tall oil fatty acids have no effect upon developmental capabilities at doses of approximately 5000 mg/kg bw/day.

It is therefore considered that 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl is unlikely to exhibit developmental toxicity. However, no data are available. Since other data gaps have been identified, as described in sections E2 and E5, a combined subchronic toxicity and reproductive / developmental toxicity screen test (OECD 422) of Westvaco DIACID® 1550 is recommended. The adoption of this combined test guideline to address the SIDS endpoints for subchronic toxicity, toxicity to reproduction and developmental toxicity is in accordance with EPA guidance (*Federal Register,* Vol 65 No 248 page 81695).

Summary of Human Health Effects (Mammalian Toxicity) Testing: Adequate data is available for bacterial and non-bacterial genotoxicity and acute oral toxicity. No data are available for subchronic toxicity, toxicity to reproduction and developmental toxicity, although data from the PCA on similar compounds suggest that 2-cyclohexene-1-octanoic acid, 5 (or 6)-carboxy-4-hexyl will have low subchronic toxicity and no developmental or reproductive toxicity. Testing of Westvaco DIACID® 1550 following the OECD 422 guideline is proposed to address these SIDS endpoints.

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III Robust Summaries of Existing Data

Physical Chemical End Point: Partition Coefficient

Test substance:
The study was carried out using the product Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
US EPA OPPTS 830.7570

GLP:
Yes

Year Study Performed:
2002

Remarks about method:
The octanol/water partition coefficient of the test material was determined using High Performance Liquid Chromatography (HPLC).

The HPLC conditions were as follows:
HPLC System: HP 1050
Column: Zorbax SB-C18 (Stable Bond), 250 mm x 4.6 mm ID, 5 micron
Mobile phase: 70:15:15 methanol:THF:pH 2 aqueous buffer (phosphate)
Wavelength: 235 nm
Flow rate: 1ml/min.

THF was used as an additive, because the test substance would not come off the column in any methanol:pH2 buffer ratio.
From preliminary work it was determined that the extinction coefficient of the test substance was considerably less than any of the reference materials. Therefore, the test substance was made up approximately 4 times the concentration (ca. 2,000 mg/L) of the reference compounds (ca. 500 mg/L). Even so, the number of area counts of the test substance was much lower than that of the reference compounds.
The test substance showed 2 closely eluting peaks, with the retention time of the test substance being calculated by averaging the 2 peaks.
Although the reference materials are supposed to bracket the material tested, the table of suggested reference materials taken from OPPTS 830.7570 had no materials with a log Kow greater than 6.2, the test substance had a calculated log Kow of 7.09.

Results:
Value of Log Kow = 7.09

Temperature:
Ambient +/-2˚C

Remarks about Results:
Surface active: Lipophilic
Dissociative: This substance can be dissociative as the HPLC system was run at pH2
Water solubility: Negligible

Conclusions:
Under the conditions of this study, DIACID 1550 had a calculated log Kow of 7.09.

Reliability:
Klimisch data reliability code 1

Remarks on Data Reliability:
Study conducted to GLP and a currently accepted guideline OPPTS 730.7570, which complies with OECD Guideline 117.

Reference:
Doi, J (2002): WESTVACO DIACID 1550, Determination of the octanol/water partition coefficient using HPLC, US EPA OPPTS 830.7570, Study No. 2002-111-005, MeadWestvaco Corporation, USA, AQUA SURVEY, INC. USA

Environmental Fate and Pathway End Point: Biodegradation

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
40CFR 796.3140

Test Type:
Anaerobic

GLP:
Yes

Year Study Performed:
1992

Contact Time:
56 days

Inoculum:
Anaerobic sludge

Remarks about method:
Inoculum: anaerobic sludge from a treatment plant, concentration of sludge in nutrient medium used was 10% level (100 ml/l)
Concentration of test chemical: 5, 10 and 20 ppm as C, aqueous solution of Westvaco DIACID® H-240 (potassium salt) in nutrient medium
Pre-acclimation conditions: None mentioned
Temperature of incubation: 35˚C (dark)
Dosing procedure: 4 replicates per test level, reference and blank
Sampling frequency: 3, 14, 28, 42 and 55 days from inoculation
Controls and blank system used: ethanol reference and sludge plus nutrient for blank
Analytical method used to measure biodegradation: gas evolution monitoring using a glass manometer
Method of calculating measured concentrations: cumulative arithmetic mean (corrected for blank) and expected theoretical yield

The gas evolution data was confirmed using organic carbon analysis.

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Degradation value = 84% within 2 months

Conclusions:
Results indicated that the sample degraded up to 84% under anaerobic conditions, based on the interpretation of both gas evolution and residual carbon analysis.

Reliability:
Klimisch data reliability code 1

Remarks on Data Reliability:
The study was conducted according to GLP and conformed to EPA Method 40 CFR 796.3140, which is equivalent to OPPTS Method 835-3400.

The testing was conducted in a large vessel (1 Litre) which allowed for the low concentrations to be measured with reasonable accuracy.

Reference:
Drozdowski D et al (1992): Anaerobic Biodegradability Testing of DIACID 1550 (6339-33) Potassium Salt, Conducted for: Westvaco Chemical Division USA, Test Report No. 064187, United States Testing Company, Inc. Biological Services USA

Environmental Fate and Pathway End Point: Biodegradation

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
OECD Method 301E

Test Type:
Aerobic

GLP:
Yes

Year Study Performed:
1991

Contact Time:
35 days

Inoculum:
Industrial sewage, adapted

Remarks about method:
Inoculum: mixed culture inoculum: rich top soil, activated sludge from a sewage treatment plant (secondary effluent) and raw surface water, inoculation of test solution with 1 ml/l of the combined inoculum
Concentration of test chemical: 19 ppm as C, aqueous test solution of Westvaco DIACID® H-240 (potassium salt) in nutrient medium,
Pre-acclimation conditions: not required by the modified OECD screening test
Temperature of incubation: 20-25˚C (in the dark, with shaking)
Dosing procedure: inoculation of each flask with 1 ml/l of combined inoculum
Sampling frequency: 0, 7, 14, 21, 28, 35 days after inoculation
Controls and blank system: aniline as reference, nutrient plus sludge as blank
Analytical method used to measure biodegradation: Acidification of supernatant sample purged with nitrogen and analyzed for DOC
Method of calculating measured concentrations: Arithmetic mean, simplified version of the OECD calculation given in method 301E

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Degradation value = 65% within 28 days

Remarks about Results:
Observed inhibition: non-inhibitory at concentrations of </= 25%
Time required for 10% degradation: 0-7 days (not specified in report), from graphical interpolation 10% degradation requires approximately 2 days
Total degradation at the end of the test: 63.2% degradation after 35 days

Conclusions:
When tested as specified Westvaco DIACID® 1550 potassium salt was not readily biodegradable, showing 65% degradation after 28 days.

Reliability:
Klimisch reliability code 1

Remarks on Data Reliability:
Study conducted to GLP and followed a currently accepted guideline.

Reference:
Drozdowski D et al (1991): Modified OECD Test for Ready Biodegradability, Shake Flask Test of Diacid 1550 Potassium Salt (6339-33), Conducted for: Westvaco Chemical Division, USA, Test Report No. 063576-3B, United States Testing Company, Inc. Biological Services Division USA

Ecotoxicity End Point: Acute Toxicity to Fish

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
40 CFR Part 797.1400

Test type:
Static

GLP:
Unknown

Year Study Performed:
1991

Species:
Pimephales promelas

Analytical method:
Gas chromatography (Flame Ionization Detector)

Exposure Period:
96 hours

Statistical Method:
Graphical interpolation

Remarks about method:
Parameters about organism:
- age: 28-31 days
- length: </= 18 mm
- weight: not known
- loading: Corrected concentrations: 0, 2.4, 4.9, 9.8, 16.3 and 41.3 ppm
- pretreatment: none, only a screening test to determine the definitive test concentrations

Parameters of Test system:
- Dilution water source: USA EPA moderately-hard reconstituted water
- Dilution water chemistry: hardness - 88 mg/l $CaCO_3$, alkalinity - 74 mg/l $CaCO_3$, pH - 7.7, Conductivity - 232 µmhos, Dissolved oxygen - 8.2 mg/l
- Stock and test solution: stock solution of the analyte Westvaco DIACID® 1550 (potassium salt 39.18% w/v) prepared at a concentration of 1075.1 ppm (activity corrected) in EPA modified hard water. Sequential dilutions were made to produce the test solutions
- Flow-through rate: not applicable as system was static and renewed daily

- Vehicle/solvent and concentrations: diluent in the system was EPA modified hard water, nominal concentrations made were 10.75, 26.75, 53.50, 80.30 and 100.00 ppm of analyte
- Stability of the test chemical solutions: considered to be stable
- Exposure vessel type: 2 L polypropylene test vessels, exposure volume 1 L
- Aeration - mixing test solutions to saturation prior to test if dissolved oxygen falls below 40% during the test oil-free air supplied at 100 +/- 10 bubble per minute
- Lighting - 16:8 hour light/dark cycle fluorescent 50-100 ft candles, 2 vessels per treatment
- Replicates: 2 replicates per treatment, 10 fish per replicate, 20 fish per treatment
- Water chemistry in test: D.O.: control - 8.2 mg/l, 16.3 ppm - 8.0 mg/l, pH: control - 7.7, 16.3 ppm - 7.7
- Test temperature range: 21 +/- 1 ˚C
- Method of calculating mean measured concentrations: arithmetic mean corrected for activity of product

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Nominal concentration:
10.75, 26.75, 53.50, 80.30 and 100.00 ppm

Measured concentration:
0, 2.4, 4.9, 9.8, 16.3 and 41.3 ppm

Endpoint:
LC50 = 15 ppm (measured) at 96 hours.

Statistical Results:
95% confidence limits not obtainable

Remarks about Results:
Biological observations: Mortality, reflex loss, erratic swim
Table showing cumulative mortality: no effects seen for concentrations 0-9.8 ppm.
For 16.3 ppm the cumulative mortality was as follows: 24 hr- 6, 48 hr- 9, 72 hr- 12, 96 hr- 15 (% mortality = 75)
For 41.3 ppm the cumulative mortality was as follows: 24 hr- 20, 48 hr - 20, 72 hr- 20, 96 hr -20 (% mortality = 100)

Lowest test substance concentration causing 100% mortality: 41.3 ppm only concentration to cause 100% mortality
Mortality of controls: controls were healthy and swam actively
Abnormal responses: none
Reference substances: none used
Any observations, such as precipitation that might cause a difference between measured and nominal values: none recorded

Additional data outside the longest end-point:
24 hr LC50 = 18.5 ppm

48 hr LC50 = 17 ppm
72 hr LC50 = 16 ppm

Conclusions:
The acute toxicity of DIACID 1550 (as potassium salt) to the freshwater minnow, *Pimephales promelas*, was found to be: 96 hour LC50 = 15 ppm (95% confidence limit not obtainable)
The No Observed Effect Concentration (NOEC) was 9.8 ppm

Reliability:
Klimisch data reliability code 2

Remarks on Data Reliability:
The study conformed to EPA Method 40 CFR 797.1400 which is equivalent to OPPTS Method 850.1075. Although there is not a GLP Compliance statement in the report, the test plan (Addendum # 3) indicate that the study was conducted according to GLP.

Reference:
Cooke D (1991): Aquatic Toxicity Tests versus *Pimephales promelas* and *Daphnia pulex* DIACID 1550, Conducted for: Westvaco Chemical Division USA, Test Report No.: 063576-2, United States Testing Company, Inc., Biological Services Division USA.

Ecotoxicity End Point: Acute Toxicity to Daphnia

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550

Chemical Category:
Fatty Acid

Method:
40CFR Part 797.1300

Test type:
static

GLP:
Unknown

Year Study Performed:
1991

Species:
Daphnia pulex

Analytical method:
Gas chromatography (Flame Ionization Detector)

Exposure Period:
48 hours

Statistical Method:
Graphical interpolation

Remarks about method:
Test organisms
- USTC stock cultures
- Age at study initiation: </= 48 hours
- Control group: diluent only

Test conditions:
- Stock solutions: vehicle/solvent: EPA moderately-hard reconstituted water, stock solution was prepared at a concentration of 1075.1 ppm (activity corrected). Sequential dilutions were made to achieve nominal concentrations of 10.75, 26.75, 53.50, 80.30 and 100.00 ppm
- Test temperature range: 22-23 ˚C
- Exposure vessel type: test vessel: 18 x 150 mm glass test tubes capped, exposure volume: 15 ml
- Aeration: aerate by mixing test solutions to saturation prior to test no aeration during test, 4 vessels per treatment.

- Dilution water source: EPA moderately-hard reconstituted water
- Dilution water chemistry: hardness: 90 mg/l $CaCO_3$, alkalinity: 80 mg/l $CaCO_3$, pH: 7.7, Conductivity: 233 µmhos, D.O.: 8.4 mg/l
- Lighting: 16:8 hour light/dark cycle, fluorescent, 50-100 ft candles (lab ambient)
- Water chemistry: D.O.: control - 8.4, 16.3 ppm - 8.4, pH: control - 7.7, 16.3 ppm - 7.8
- Endpoints assessed: immobilization
- Test design: 4 replicates per treatment, 5 daphnia per replicate, 5 concentrations (measured) 2.4, 4.9, 9.8, 16.3 and 41.3 ppm
- Method of calculating mean measured concentrations arithmetic mean corrected for activity

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Nominal concentration:
10.75, 26.75, 53.50, 80.30 and 100.00 ppm

Measured concentration:
2.4, 4.9, 9.8, 16.3 and 41.3 ppm

Endpoint:
LC50 = 22 ppm (measured) at 48 hours.

Statistical Results:
95% confidence limits not obtainable

Remarks about Results:
Biological observations

- Number immobilized as compared to the number exposed: 0/20 for 2.4, 4.9 & 9.8 ppm, 07/20 at 16.3 ppm, 18/20 at 41.3 ppm
- Concentration response with 95% confidence limits: not obtainable
- Cumulative immobilization: 16.3 ppm: 24 hr - 2, 48 hr - 5 (% mortality 25), 41.3 ppm: 24 hr - 7, 48 hr - 18 (% mortality 90)
- Control response: satisfactory

Additional data outside the longest end-point:
24hr LC50 = >41.3 ppm (95% C.L. not obtainable)

Conclusions:
The acute toxicity of Westvaco DIACID® 1550 (as potassium salt) to the water flea, *Daphnia pulex*, was found to be:
48 hour LC50 = 22.5 ppm (95% confidence limit was not obtainable)
The No Observed Effect Concentration (NOEC) was 9.8 ppm

Reliability:
Klimisch data reliability code 2

Remarks on Data Reliability:
The study conformed to EPA Method 40 CFR 797.1300 which is equivalent to OPPTS Method 850.1010. Although there is not a GLP Compliance statement in the report, the test plan (Addendum # 3) indicates that the study was conducted according to GLP.

Reference:
Cooke D (1991): Aquatic Toxicity Tests versus *Pimephales promelas* and *Daphnia pulex* DIACID 1550, Conducted for: Westvaco Chemical Division, USA, Test Report No. 063576-2, United States Testing Company, Inc. Biological Services Division, USA

Ecotoxicity End Point: Acute Toxicity to Algae

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550.

Chemical Category:
Fatty Acid

Method:
40CFR Part 797.1050

Test type:
static

GLP:
Yes

Year Study Performed:
1991

Species:
Selenastrum capricornutum

Endpoint:
Growth rate, cell count by hemocytometer

Analytical method:
Total organic carbon analysis (Ionics 1555)

Exposure Period:
96 hours

Statistical Method:
Probit analysis

Remarks about method:
Test organisms
- Laboratory culture: USTC Stock Culture, origin EPA, Cincinnati, Ohio
- Method of cultivation: incubation in appropriate media, at 20 +/- 2 ˚C under 400 +/- 50 ft. candles using a 16:8 light/dark photoperiod, with manual shaking periodically
- Controls: vessels containing algal medium were inoculated and then incubated as for test vessels

Test Conditions
- Test temperature range: 24 +/- 2 ˚C
- Growth/test medium chemistry: pH: 7.4, alkalinity: 30 mg/l $CaCO_3$, hardness: 50 mg/l $CaCO_3$, conductivity: 130µmhos
- Dilution water source: OECD test medium

- Exposure vessel type: 125ml Erlenmeyer Flasks, 50 ml solution volume
- Aeration: mixing test solutions to saturation prior to test no aeration during test, 3 replicates per treatment
- Water chemistry in test: 32 ppm - pH 7.3/7.5 (0/96 hrs), 63 ppm - pH 7.3/7.4 (0/96 hrs), 125 ppm - pH 7.2/7.3 (0/96 hrs), 250 ppm - 7.1/7.2 (0/96 hrs), 500 ppm - 7.1/7.2 (0/96 hrs)
- Stock solutions preparation: 5 g/195 ml test material in test medium, 10,000 and 1,000 ppm, serial dilutions to: 32, 63, 125, 250 and 500 ppm
- Light levels and quality during exposure: continuous light, fluorescent 400-450 ft candles

Test design: 3 replicates per treatment, inoculum density 10,000 cell per ml
Test concentrations: 32, 63, 125, 250 and 500 ppm
Method of calculating mean measured concentrations: Total organic carbon count

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Nominal concentration:
32, 63, 125, 250, 500 ppm

Measured concentration:
32, 50, 500 ppm confirmed, 63, 125 ppm variable

Endpoint:
EC50-CD = 88 ppm (nominal) at 96 hours
NOEC <= 32 ppm (nominal) based on growth inhibition
LOEC = 40 ppm (nominal) based on growth inhibition.

Statistical Results:
At 96 hours EC point 95% confidence limits upper/lower: EC1- 2.5/39, EC5- 6.7/52, EC10- 11/62, EC15- 16/69, EC50- 55/137, EC85- 112/463, EC90- 126/647, EC95 - 149/1076, EC100- 201/2850

Remarks about Results:
Response of control group satisfactory
Biological observations
- Cell density (per ml of x 10-E4) at each flask at each measuring point: control - 23, 25, 26 (48 hrs) 118, 130, 125 (72 hrs) 180, 194, 200 (96 hrs), 32 ppm- 23, 23, 20 (48 hrs) 95, 97, 80 (72 hrs) 202, 210, 212 (96 hrs), 63 ppm - 22, 19, 18 (48 hrs) 65, 70, 73 (48 hrs) 120, 125, 120 (96 hrs), 125 ppm - 16, 19, 14 (48 hrs) 23, 24, 24 (72 hrs) 45, 37, 38 (96 hrs), 250 ppm 13, 13, 13 (48 hrs) 12, 14, 15 (72 hrs) 20, 19, 18 (96 hrs), 500 ppm < inoculum at each time point
- Growth curves: log plot of cell number vs hrs for each concentration, showed reduced growth at all levels, lower levels produced smaller % inhibition
growth rate inhibition per concentration 48/72/96 hrs: 32 ppm - 12/27/0%, 63 ppm - 20/44/36%, 125 ppm - 36/81/79%, 250 ppm - 48/89/79%, 500 ppm - 100/100/100%
- Observations: Cell growth was insufficient at 24 and 48 hours to establish concentration-effect relationships for all concentrations. Subculturing of cells from

exposure concentrations recovered viable cells, demonstrated by resumption of growth where previously retarded. Test concentrations up to 500 ppm were thus algistatic rather than algicidal. Morphological changes, as smaller cell size were seen at 250 and 500 ppm. It was possible that sample degradation occurred under test conditions, thus exposure to the test material should not be considered to be uniform throughout the 96 hr test period, but only to concentrations established at the onset of testing.

Conclusions:
For Westvaco DIACID® 1550 (as potassium salt) on the basis of 100% active sample.
96 hr EC50 = 87.6 ppm (95% C.L. = 54.5-137.4 ppm)
96 hr EC10 = 39.9 ppm
96 hr EC90 = 192.6 ppm
NOEC approximately 32 ppm.

Test concentrations up to 500 ppm proved that test material was algistatic.

Reliability:
Klimisch data reliability code 1

Remarks on Data Reliability:
The study was conducted according to GLP and conformed to EPA CFR 40 Part 797.1050 which is equivalent to currently accepted OPPTS Method 850.5400 (which follows the general principles of OECD Method 201)

Reference:
Drozdowski D (1991): Algal Acute Toxicity Test of DIACID 1550 Potassium salt (6339-33), Conducted for: Westvaco Chemical Division USA, Test Report No. 063576-3A, United States Testing Company, Inc., Biological Services Division, USA.

Toxicity End Point: Acute Toxicity

Test substance:
The study was carried out using the product Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
No specific guideline is quoted

GLP:
No

Year Study Performed:
1973

Species:
rat

Strain:
Sprague-Dawley

Sex:
Both

Number of males per dose:
2

Number of females per dose:
2

Vehicle:
corn oil, 25% w/v/ suspension

Route of administration:
Oral – by intubation syringe

Remarks about method:
The animals used were classed as 'young'
There were 4 dose groups and animals received a single dose
Concentration administered: 3038; 4556; 6834 and 10250 mg/kg
There was a 14 day post dose observation period

End Point:
Acute lethal value = 6176 mg/kg bw

Deaths per dose:

Deaths occurred in 0/4, 0/4, 3/4 and 4/4 rats treated at 3038, 4556, 6834 & 10250 mg/kg respectively

Remarks about Results:
Rats dosed at 6834 mg/kg died between 6-22 hours and 2 days after dosing; 1 female rat 6-22 hours after dosing and 1 male and 1 female rat died 2 days after dosing.
Rats dosed at 10250 mg/kg died between 6-22 hours and 2-3 days after dosing; both females died 6-22 hours after dosing and 1 male died 2 days after dosing and the remaining male died 3 days after dosing.

Clinical signs at 3038 mg/kg: hypoactivity and ruffed fur which onset 1 hour after dosing with a duration of 1 day.
Clinical signs at 4556 mg/kg: hypoactivity and ruffed fur which onset 1 hour after dosing with a duration of 2 days, laboured breathing onset after 3 hours with a duration of 6-22 hours.
Clinical signs at 6834 mg/kg: hypoactivity and ruffed fur which onset 1 hour after dosing with a duration of 5 days, laboured breathing onset after 2 hours with a duration of 2 days. Muscular weakness onset 3 hours after dosing with a duration of 2 days. Diuresis onset 6-22 hours after dosing with a duration of 2 days.
Clinical signs at 10250 mg/kg: hypoactivity and ruffed fur which onset 1 hour after dosing and laboured breathing and muscular weakness onset after 2 hours Diuresis onset 6-22 hours after dosing with a duration for all signs until death.

All animals that died underwent necropsy examination and revealed gastroenteritis. No gross pathologic alterations were noted among the animals sacrificed at the end of the 14 day observation period.

Conclusions:
The LD50 is 6176 mg/kg bw for acute oral toxicity to rats.

Reliability:
Klimisch data reliability code 2

Remarks on Data Reliability:
The study was not conducted according to any recognised guideline and only 4 animals per dose group were administered the compound. The observation period was appropriate for an acute study and generally recognised procedures appear to have been followed. Although the study does not comply with any acceptable guidelines (past or current) in the interests of animal welfare, these results should be considered acceptable.

Reference:
Hintz C *et al*. (1973): Report to Westvaco: Acute toxicity studies with DIACID 1550, P.O. No. S-10590, IBT No. 601-04128, Industrial BIO-TEST Laboratories, Inc.

Toxicity End Point: Bacterial Gene Mutation

Test substance:
The study was carried out using the product Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
Method used followed all procedures outlined by Ames – Ames test

GLP:
Yes

Year Study Performed:
1991

Species:
Salmonella typhimurium Strains used were TA 98, 100, 1535, 1537 and 1538

Metabolic Activation:
activator mammalian liver S-9, with or without

Concentration:
10 mg/ml, 100 mg/ml in Dimethylsulfoxide

Statistical Method:
The average number of revertant colonies (+/- SD)

Remarks about method:
Test Design
- Number of replicates: 2 plates for 10 mg/ml and 3 plates for 100 mg/ml, plus 1 solvent and 1 positive control, with activation and the same without activation per strain
- Frequency of Dosing: one dosing only, followed by a 48 hour incubation period
- Positive and negative control groups and treatment: 1 positive and 1 negative control group and two treatment groups with and without activation per strain

Solvent/vehicle: solvent used was dimethylsulfoxide a concentration of 10 and 100 mg/ml of test material

Criteria for evaluating results: spontaneous reversion frequency is measured. A test material producing a consistent bacterial response twice that of the solvent or spontaneous reversion count is indicated as positive for TA 98 and 100, a three times response is indicated as positive for TA 1535, 1537 and 1538

Results:
Negative

Cytotoxic concentration:

100 mg/l

Genotoxic Effects:
Unconfirmed

Statistical Results:
None, average (+/- SD) revertant colony counts for all strains at both concentrations and with or without activation were comparable to the solvent control in all cases.

Remarks about Results:
The test material had a maximum solubility in dimethylsulfoxide of 100 mg/ml and is sparingly soluble in water

Conclusions:
When tested as specified Westvaco DIACID® 1550 did not exhibit mutagenicity versus test strains TA 98, 100, 1535, 1537 and 1538 in the presence or absence of activation at maximum solubility and cytotoxicity.

Reliability:
Klimisch reliability code 1

Remarks on Data Reliability:
The test was not carried out according to currently accepted guidelines, but it was conducted to GLP and followed a method which is still referenced in current guidelines.

References:
Tong C C (1991): Ames Mutagenicity Testing of DIACID 1550, Conducted for: Westvaco Chemical Division, USA, Test Report No. 063576-1B, United States Testing Company, Inc, Biological Services Division, USA,

Ames et al (1975): Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity test, Mutation Research, 31, 347-364

Toxicity End Point: In vitro gene Mutation

Test substance:
The study was carried out using the product Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
OECD 473 Chromosomal aberration in cultured Chinese hamster ovary cells

GLP:
Yes

Year Study Performed:
1991

Species:
Chinese Hamster Ovary cells

Metabolic Activation:
S-9 activation system 7.5 ml/100 ml (S-9 to culture medium)

Concentration:
non-activated: 13, 25, 50, 75, 100, 150 µg/ml, activated: 6.3, 13, 25, 50, 75, 100 µg/ml

Statistical Method:
Chi-square test for positive controls only

Remarks about method:
Test Design

- Number of replicates: 4 per dose level, 2 for cytotoxicity test and 2 for chromosome aberration assay
- Frequency of Dosing: 1 dose, exposure to test material for 2 hours with activation and 16 hours without activation followed in both cases by incubation and additional harvesting time
- Positive controls - triethylenemelamine (0.5 µg/ml) non-activation and cyclophosphamide (30 µg/ml) activation
- Number of metaphases analyzed for chromosomal studies: 100 metaphases at each dose level (50 per duplicate group). Only cells showing 18-20 chromosomes were scored.

Solvent used was dimethylsulfoxide (DMSO), at a maximum of 1% of the volume of the culture medium
This main study was preceded by a range-finding study with concentrations from 0.1-5000 µg/ml (10 levels)
Number of metaphases analyzed for chromosomal studies: 100 metaphases at each dose level (50 per duplicate group). Only cells showing 18-20 chromosomes were scored

Results:
Negative

Cytotoxic Concentration:
At 100 µg/ml the Relative Cell Growth (RCG) was reduced to 54% (non-activated) and 13% (activated)

Genotoxic Effects:
Unconfirmed

Statistical results:
Chi-square test was used on positive controls, results were considered significant if p is </= 0.05. There were no statistically significant results in the test doses.

Remarks about Results:
Miscibility was tested during the range-finding test and indicated the test article formed a turbid suspension with water. There was precipitate in the medium in the test flasks at concentrations of 500, 1000 and 5000 µg/ml in DMSO, indicating miscibility limitations at this level. However, since cells did not survive at these doses, thus these levels were not used in the main study. Osmolality values were determined during the range-finding test, again at levels 500, 1000 and 5000 µg/ml precipitate was visible in the medium. However, none of the doses tested showed an osmolality beyond 427 (mOsmol/kg water) which was below the range that may cause damage to the cells.

There were no dose-related effects seen. In the non-activated system there were no scorable metaphases at 150 µg/ml, chromosome aberrations were scored from controls and 25, 50, 75 and 100 µg/ml. In the activated system there were insufficient scorable metaphases at 100 µg/ml, chromosome aberrations were scored from 13, 25, 50 and 75 µg/ml.

No. of aberrations per cell (non-activated) 0.01-0.03 for 25-100 µg/ml, </= to solvent values.
No. of aberrations per cell (activated) 0.01-0.02 for 13-75 µg/ml, < solvent values

Conclusions:
Under the conditions of this study, Westvaco DIACID® 1550 did not induce a significant increase in chromosome aberrations nor was there any indication of a positive dose trend in either the non-activated or activated systems. Therefore the test article is considered to be negative in the in-vitro CHO chromosome aberration assay.

Reliability:
Klimisch reliability code 1

Remarks on Data Reliability:
Study conducted to GLP and accepted guideline.

Reference:

Kumaroo, P V (1991): Test for Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation, Westvaco Chemical Division, USA, Study No. 0172-3110, Sitek Research Laboratories, USA