

Environmental Assessment for
Rumen-Stable, Abomasum Dispersible Amino Acids
for Dairy Cattle and Dairy Replacement Heifers

FAP 2231

1. Date: May, 1994
2. Name of Petitioner: Rhone Poulenc Animal Nutrition
3. Address: 42, Avenue Aristide Briand
B.P. 100
92164 Antony Cedex, France

4. Description of the Proposed Action

a. The Purpose and Need for the Action

The purpose of this petition is to secure a food additive regulation which will enable the petitioner to offer the amino acids, methionine and lysine, for dairy cattle and dairy replacement heifers in a form which will protect these articles from destruction in the rumen but will, by use of a specialized polymeric substance, poly (2-vinyl-pyridine-co-styrene) (copoly VP/ST), permit their transport through the rumen into the abomasum where the polymer loses its integrity releasing the nutrients for absorption. The polymer is produced by classical emulsion polymerization procedures, dried, and applied by suitable means to the nutrient to be protected.

b. The Locations Where the Product Will Be Produced

The polymer will be produced and purified to meet established specifications at Rhone Poulenc Animal Nutrition, Commentry, France, and employed in the production of rumen-protected lysine and methionine (RPAA) at Rhone Poulenc Animal Nutrition, Montlucon, France.

c. The Locations Where the Product Will be Used

The RPAA will be marketed throughout the United States for use as a nutrient supplement in the feed of dairy cattle.

RPAA is approved for use as a nutrient supplement in the feed of dairy cattle within the EEC Commission Directive 82/471/EEC of 4 June 1993, Official Journal of the European Communities, Vol. 36, L179.

002573

d. The Location Where the Product Will Be Disposed Of

The polymer is not absorbed by the ruminant animal but is excreted, chemically unchanged, in the feces with other waste products.

e. The Types of Environments Present at Locations Identified at b, c and d

The environment at Rhone Poulenc Animal Nutrition, Commentry, France, where copoly VP/ST will be produced, is characteristic of a highly diversified chemical and vitamin manufacturing facility. The environment at Rhone Poulenc Animal Nutrition, Montlucon, France, where copoly VP/ST will be used in the manufacture of RPAA, is characteristic of a highly specialized manufacturing facility. Consequent activities involving RPAA will occur wherever dairy cattle are fed for production of milk. Feces generated by these animals will be distributed to the terrestrial environment as manure according to common agricultural practices.

5. Identification of Chemical Substances Subject to the Proposed Action

a. Introduction

Rumen-Stable Amino Acids will be supplied as pellets produced from methionine and lysine with the adjuvant substances, edible fatty acids, i.e., stearic acid, and copoly VP/ST. These pellets will be coated or enrobed with a formulation containing copoly VP/ST and edible fatty acids.

A typical rumen-protected amino acid preparation has the following approximate composition:

<u>Component</u>	<u>Percent</u>
Lysine Hydrochloride	50.0
Methionine	15.0
Stearic Acid	30.0
Copoly VP/ST	3.0
Ethyl cellulose	2.0

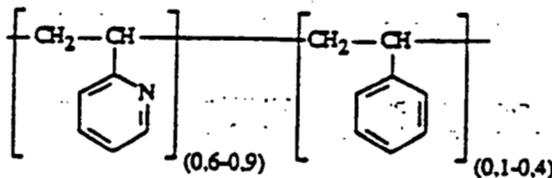
The final product will contain less than 6.0 ppb each of the monomers, styrene and 2-vinylpyridine. (One specification for the polymer is that it contain less than 200 ppb of each monomer.) The value 6.0 is the fraction of the polymer in a typical final product (0.03) multiplied by 200 ppb.

002574

b. Poly (2-vinylpyridine-co-styrene)

CAS Registry Number: 24980-54-9

Structural formula:



The copolymer is a random arrangement of repeating units derived from 2-vinylpyridine and styrene. The probabilities of various sequences along the polymer chain are determined by the relative reactivities of the monomers and their relative concentrations during polymerization. In the final polymer, the 2-vinyl-pyridine: styrene ratio is between 65:35 and 80:20.

Molecular weight: 100,000-5000,000

Solubility: <0.1% in corn oil; between 0.1% and 1.0% in acetone, dimethyl sulfoxide, octanol, tetrahydrofuran, and p-dioxane; in water, 92.7 ppb at pH 5, 88.6 ppb at pH 7, and 185.6 ppb at pH 9.^{1,2}

Vapor pressure: Estimated by the CHEMEST³ Program to be 1.94×10^{-26} Torr (appendix D).

Octanol-water partition coefficient²: 10^4 to 10^5 .

Soil absorption coefficient: 1.08×10^5 (Appendix D).

Ultraviolet absorptivity: 23.96 at 262 nm.⁴

c. Stearic Acid (as an example of an edible fatty acid)⁶

CAS Registry Number 57-11-4

Structural formula: $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$

Solubility: very slightly soluble in water; 1% soluble in alcohol, acetone, chloroform, carbon disulfide and other organic solvents.

Boiling point: 383°C.

Melting point: 69-70°C.

002575

d. Methionine⁷

CAS Registry Number: 63-68-3

Structural formula: $\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$

Solubility: in water, 18.8 g/L at 25°C, 33.81 g/L at 50°C; very slightly soluble in 95% alcohol; insoluble in ether.

Melting point: 281°C (dec.)

e. Lysine Hydrochloride⁸

CAS Registry Number: 56-87-1

Structural formula: $\text{NH}_2(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{COOH}$

Solubility: freely soluble in water; very slightly soluble in alcohol; insoluble in ether.

Melting point: 210-224.5°C (dec.)

f. Styrene⁹

CAS Registry Number: 100-42-5

Structural formula: $\text{C}_6\text{H}_5\text{CH}=\text{CH}_2$

Solubility: in water, 280 mg/L at 15°C, 300 mg/L at 30°C, 400 mg/L at 40°C.

Octanol-water partition coefficient: 891 (Log K_{ow} = 2.95)
(Appendix 2)

Soil absorption coefficient: 573 (Appendix 2)

Ultraviolet absorbance: Molar extinction coefficient = 15140 at 245 nm in ethanol.⁴

Boiling point: 145.2°C

Vapor pressure: 10 mm at 30.8°C

Melting point: -30.63°C

002570

g. 2-Vinylpyridine¹⁰

CAS Registry Number: 100-69-6

Structural formula: $C_5H_4NCH=CH_2$

Solubility: in water, 27.5 g/L at 20°C; very soluble in alcohol, acetone, ether, and other organic solvents.

Octanol-water partition coefficient: 63 (Log K_{ow} = 1.80)
(Appendix 3)

Soil adsorption coefficient: 47 (Appendix 3)

Ultraviolet absorbance: Molar extinction coefficient = 12600 at 238nm in ethanol.⁴

Boiling point: 159-160°C

Vapor pressure: 10 mm at 44.5°C

6. Introduction of Substances Into the Environment:

a. Assumptions for amounts of materials entering the environment.

- 1). RPAA will be marketed to dairy cattle and dairy replacement heifers.
- 2). The 1993 U.S. dairy cow population is 9.8 million and the dairy replacement heifer population is 4.9 million.
- 3). All dairy cattle in the U.S. will receive RPAA.
- 4). The amount of RPAA fed per head per day is up to 50 g.

The actual amount will vary according to the ration. Typical feeding rates range from five up to 50 g per head per day. Twenty five grams will be considered a typical dose.

- 5). RPAA will be fed for 365 days/year.

b. Controls Used at the Manufacturing Site

The finished rumen protected amino acid product is manufactured at Rhone Poulenc, Montlucon, France. Copoly VP/ST as a dry powder is

002577

blended with edible fatty acids and the blend is combined with the appropriate amino acids and compounded into nutrient cores. Compounding is by extrusion of the dry components followed by cutting and rounding. Fines produced as a result of this operation are retained on filters for collection and recycled. The cores are coated with an aqueous solution of copoly VP/ST and stearic acid.

c. Quantity and Concentration of Substances Expected to Enter the Environment through Feeding RPAA to Dairy Cattle:

The purpose of RPAA is to deliver the active ingredients, methionine and lysine, past the rumen of dairy cattle where upon the polymer (copoly VP/ST) coating and matrix loses its integrity releasing the protected nutrient.

Assumptions:

- 1). Number of dairy cattle and dairy replacement heifers in the U.S. = 9.8 million and 4.9 million, respectively.
- 2). Amount of RPAA fed = 25 g/head/day and 10 g/head/day for cows and heifers, respectively.
- 3). Feeding period = 365 days and 200 days for lactating cattle and replacement heifers respectively.
- 4). The two amino acids and the edible fatty acid used in the production of RPAA are absorbed by the ruminant.
- 5). All copoly VP/ST and trace quantities of styrene and 2-vinylpyridine enter the environment in the feces of dairy cattle fed RPAA.
- 6). Manure application rate on a dry basis is 10.23×10^3 kg/acre/year. According to Dr. Charles Strasia, Oklahoma State University Extension Center, the moisture content of air-dry manure that is normally spread is 25%.¹⁵ His estimated application rates agree with the value of 10 to 15 air-dried tons per acre that is given in The Feedlot.¹⁶ The calculation for manure application rate on a dry basis is: 15 air-dried tons/acre x .75 (the fraction of dry manure in air-dried manure) x 2000 lb/ton/2.2 lb/kg gives the amount of manure per acre on a dry basis in kilograms.
- 7). a) Dairy cattle produce approximately 36 to 41 kg of manure per day that is about 80% moisture or 8.2 kg of dry manure per head per day

002578

- b) Dairy replacement heifers produce approximately 15 kg of manure per day that is about 80% moisture or 3 kg of dry manure per head per day.

8). Weight of an acre-furrow¹⁷ = 9.90×10^5 kg.

The composition of RPAA given earlier in this Environmental Assessment is repeated here for the convenience of the reader.

<u>Component</u>	<u>Percent</u>
Lysine Hydrochloride	50.0
Methionine	15.0
Stearic Acid	30.0
Copoly VP/ST	3.0
Ethyl cellulose	2.0

The final product will contain less than 6.0 ppb each of the monomers, styrene and 2-vinylpyridine. (One specification for the polymer is that it contain less than 200 ppb of each monomer.) the value 6.0 ppb is the fraction of the polymer in the final product times 200 ppb (0.03×200 ppb).

Quantity and Concentration of Copoly VP/ST per year:

Fraction Copoly VP/ST in RPAA = 0.03

Quantity of Copoly VP/ST = 6.6M lb/year

$$(9.8 \times 10^6 \text{ cows} \times 25 \text{ g RPAA/head/day} / 454 \text{ g/lb} \times 365 \text{ days} \times 0.03 = 5.91\text{M lb/year})$$

$$4.9 \times 10^6 \text{ heifers} \times 10 \text{ g RPAA/head/day} / 454 \text{ g/lb} \times 200 \text{ days} \times 0.03 = 0.65\text{M lb/year}$$

Quantity of RPAA = 220M lb/year

$$(6.6\text{M lb/year} / 0.03 = 183.3\text{M lb/year})$$

Concentration in manure = 9.4×10^{-5} kg/kg of manure, weight average

$$\text{For dairy cattle } (0.03 \times 25 \text{ g/head/day} / 1000 \text{ g/kg} / 8.2 \text{ kg manure/head/day} = 9.1 \times 10^{-5} \text{ kg/kg manure})$$

002579

For heifers, (0.03×10 g/head/day/1000 g/kg/3.0 kg manure/head/day = 1.0×10^{-4})

Weighted average = 9.8×10^6 dairy cows $\times 9.1 \times 10^{-5} = 891.8$

4.9×10^6 heifers $\times 1 \times 10^{-4} = 490.0$

$1381.8 \div 1.47 \times 10^7 = 9.4 \times 10^{-5}$ kg/kg manure

Concentration in soil = 1.06×10^{-6} kg/kg soil or 1.06 mg/kg

$(9.4 \times 10^{-5}$ kg copoly VP/ST/kg manure $\times 10.23 \times 10^3$ kg manure/acre/year/ 9.09×10^5 kg/acre-furrow = 1.06×10^{-6} kg/kg soil

Quantity and Concentration of Styrene and 2-Vinylpyridine each per year:

Concentration of styrene and 2-vinylpyridine each in RPAA = 6.0 ppb.

Quantity - 1.31 lb styrene and 2-vinylpyridine each/year

For dairy cattle (9.8×10^6 head $\times 25$ g/head/day/454 g/lb $\times 365$ days $\times 6.0 \times 10^{-9} = 1.18$ lb of each monomer)

For heifers (4.9×10^6 head $\times 10$ g/head/day/454 g/lb $\times 200$ days $\times 6 \times 10^{-9} = 0.13$ lb of each monomer)

Concentration in manure = 1.9×10^{-11} kg (19 ppt) styrene and 2-vinylpyridine each per kg of manure

For dairy cattle ($6.0 \times 10^{-9} \times 25$ g/head/day/1000 g/kg/8.3 kg manure/head/day) = 1.83×10^{-11} kg/kg manure)

For heifers ($6 \times 10^{-9} \times 10$ g/head/day/1000 g/kg/3.0 kg manure/head/day - 2.0×10^{-11} kg/kg manure)

Concentration in soil = 2.1×10^{-13} kg (0.21 ppt) styrene and 2-vinylpyridine each/kg of soil

$(1.9 \times 10^{-11}$ kg/kg manure $\times 10.23 \times 10^3$ kg manure/acre/year/ 9.09×10^5 kg/acre-furrow = 2.1×10^{-13} kg/kg of soil or 0.21 parts per trillion)

7. Fate of Emitted Substances in the Environment

Since the expected route of introduction of copoly VP/ST into the environment is through excretion by the target animal, the petitioner

002580

conducted several studies of the fate of this food additive in the environment. Reports of specific fate tests, identified below, along with the protocols submitted to the administration for acceptance prior to initiating the tests, are included as Heading H, Enclosure 6, Parts A-G.

Styrene and 2-Vinylpyridine Fate:

Styrene and 2-vinylpyridine have appreciable vapor pressures, 10mm at 30.8°C and 44.5°C respectively. Therefore, the 1.16 lb/year (0.21 ppt) of each that is expected to enter the environment is expected to volatilize into the air. Assuming that the monomers are continuously released from a single point source, and utilizing the technique for approximating the impact of area sources outlined in Turner's workbook¹⁸, the predicted maximum ambient concentrations of styrene and 2-vinylpyridine are 57 ppt and 61 ppt respectively. This methodology predicts the highest possible concentration of monomers in ambient air.

These maximum concentrations were calculated as follows:

An approximation of an area sources ambient impact can be made by combining all of the emissions in a given area and treating this area as a source having an initial horizontal standard deviation, σ_{y0} . A virtual distance, xy , can then be found that will give this standard deviation. Values of xy will vary with atmospheric stability. then equations for point sources may be used, determining σ_y as a function of $x + xy$.

This procedure treats the area source as a cross-wind line source with normal distribution, a fairly good approximation for the distribution across an area source. The initial standard deviation for a square area source can be approximated by $\sigma_{y0} = s/4.3$, where s is the length of a side of the area.

The following calculations illustrate the application of this technique to predict the maximum downwind concentrations of styrene and 2-vinylpyridine:

$$\begin{aligned}\text{area of source} &= 4.1 \times 10^6 \text{ acres} \\ &= 1.8 \times 10^{11} \text{ sq. ft}\end{aligned}$$

assuming the area is square;

$$\begin{aligned}\text{length of side, } s &= 4.2 \times 10^5 \text{ ft} \\ &= 1.3 \times 10^5 \text{ meters}\end{aligned}$$

002581

emission rate, $q = 0.26$ g/s for styrene
 $= 0.28$ g/s for 2-vinylpyridine

Note: Emission rates are based on convection mass transfer calculations.

$$\sigma_{y0} = s/4.3 = 3.0 \times 10^4 \text{ meters}$$

maximum ground level concentration,
 $C_{\max} = q/(3.14159 \times \mu \times \sigma_{y0} \times \sigma_{z0})$

where:

q = emission rate, g/s

μ = wind speed, m/s

$\sigma_{y0} \times \sigma_{z0}$ = horizontal and vertical standard deviations of plume concentration distribution, m.

assuming a neutral atmospheric stability and a 5 mph wind;

$$\sigma_{z0} = 5 \text{ meters}$$

$$\mu = 2.24 \text{ m/s.}$$

$$C_{\max} = 57 \text{ ppt for styrene}$$
$$= 61 \text{ ppt for 2-vinylpyridine}$$

Copoly VP/ST Fate in specific compartments:

a. Air

Copoly VP/ST is a polymer having a molecular weight in the range 100,000 to 500,000, and an estimated vapor pressure of 1.94×10^{-26} Torr. Therefore, it is not expected to enter the air during manufacture or use.

b. Aquatic Ecosystems

Because copoly VP/ST has a low solubility in water the use and disposal of copoly VP/ST and RPAA will not result in any direct release of copoly VP/ST to the aquatic environment. the solubility and bioconcentration information that follow indicate that no more than trace quantities of copoly VP/ST are likely to enter the aquatic environment and that no subsequent bioconcentration is expected to occur.

002582

Organic Solvent Solubility

The petitioner has examined the general solubility of copoly VP/ST in distilled water, corn oil, acetone, octanol, dimethylsulfoxide (DMSO), tetrahydrofuran (THF), and p-dioxane. Visual inspection was used to determine whether or not copoly VP/ST was completely dissolved by each of these seven common solvents following sonication of 0.1, 1.0, and 10% mixtures of polymer in these solvents. The polymer has a low solubility (<0.1%) in distilled water, corn oil, and acetone; it has a moderate solubility (0.1% S 1.0%) in DMSO, octanol, THF, and p-dioxane.¹

Aqueous Solubility

In a more definitive study,² the solubility of copoly VP/ST was measured in aqueous solutions at pH values of 5, 7, and 9. A sample of (¹⁴C) copoly VP/ST having the same specifications as commercial copoly VP/ST was used. (¹⁴C)Copoly VP/ST was added to twelve 125-mL Hypo-Vials®. Six of the vials received approximately 50 mg each of (¹⁴C)Copoly VP/ST and six received approximately 100 mg each. Two vials at each weight received either 50 mL acetate buffer (pH 5), 50 mL phosphate buffer (pH 7), or 50 mL borate buffer (pH 9). One sample of each weight at each pH was sonicated for 0.25 minutes and one sample of each weight at each pH was sonicated for 30 minutes. All samples were then incubated with shaking overnight at 25°C. Each sample was then centrifuged at 9,000 x g for ten minutes. One mL (in triplicate) of the aqueous phase was mixed with 10 mL of Eastman Ready-to-Use II® scintillation cocktail. The samples were cooled and ark adapted for four hours, then counted for 20 minutes each in a Packard Model 4530 Liquid Scintillation Spectrometer. A calibration curve was prepared using samples of (¹⁴C) copoly VP/ST in pyridine. Using linear regression analysis, the equation for the calibration curve was:

$$y = 1.0137 x - 40.55$$

where y = net sample dpm and x = (¹⁴C)copoly VP/ST concentration. the correlation coefficient = 0.9987. the net sample dpms were inserted into this equation and solved for the concentration of copoly VP/ST.

The mean solubility of (¹⁴C)copoly VP/ST was determined to be as follows: pH 5, 92.7 ppb; pH 7, 88.6 ppb; and pH 9, 185.6 ppb.² Copoly VP/ST is not expected to leach from soil because of the extremely low aqueous solubility, an octanol-water partition coefficient of 10^4 to 10^5 ,

002583

and a soil absorption coefficient estimated to be approximately 10^5 (Appendix 1).

Bioconcentration Potential

A large octanol-water partition coefficient may indicate that a compound will bioaccumulate in organisms in the environment. However, this coefficient is not considered to be an accurate indication of bioconcentration potential for copoly VP/ST. First, it is known that high molecular weight compounds are not appreciably absorbed in the G. I. tract. For example Tagesson, et al, studied the intestinal permeability of the water soluble poly (ethylene glycol) (PEG) in the molecular weight range 634-1338. An exponential decrease was observed with the highest molecular weight showing less than 2% absorption.¹⁹ The much higher molecular weight for copoly VP/ST should further decrease its absorption. Another factor that should cause a very low or negligible passage of this polymer through cell membranes is its extremely low aqueous solubility.

These arguments are supported by the results of a study in which carbon-14 labeled copoly VP/ST was fed to rats.²⁰ In this study, instead of 2-vinylpyridine, 2-methyl-5-vinylpyridine with similar molecular weight, mol. wt. distribution, and chemical and physical properties was used. The FDA accepted data from this polymer as being applicable to the 2-vinyl case. This polymer was also called copoly VP/ST.

Carbon-14 labeled copoly VP/ST was dissolved in acetone and administered by gavage to male Charles River rats (N=3) at a dose of 100 mg/kg of body weight. Essentially all of the radioactivity administered was recovered in the feces within 72 hours after dosing. Radioactivity in the carcass, urine, blood or tissues, with the exception of the gastrointestinal tract, was below the limit of detection. The total recovery of carbon-14 ranged from 100 to 107% of the administered dose. The analysis of fecal extracts by thin layer chromatography suggested that the radioactive material in the feces was similar to that of an authentic sample of carbon-14 labeled polymer. These findings suggest copoly VP/ST was stable in the gastrointestinal tract, was not absorbed, and was eliminated in the feces unchanged.²⁰

Therefore, the potential for copoly VP/ST to bioconcentrate is considered to be low.

Biodegradation

A shake-flask biodegradation study, designed to estimate the extent to which copoly VP/ST will be degraded in surface waters and by biological waste treatment microorganisms, was conducted.²¹

The microbes used in this study were from a laboratory culture and were acclimated to copoly VP/ST for 21 days prior to the start of the test. Acclimation consisted of introducing into the culture (every two to three days) progressively less food and progressively more polymer. After acclimation, quintuplicate samples of about 11 mL each were prepared in biometer flasks in the following manner.

Treatment

<u>Sample</u>	<u>BSM</u>	<u>SA</u>	<u>TC</u>	<u>THF</u>	<u>Inoc.</u>	<u>Gluc</u>
Test Solution	10 mL		50 μ L		1 mL	
Sterile Control	10 mL	1 mL	50 μ L			
Negative Control	10 mL			50 μ L	1 mL	
Positive Control	10 mL				1 mL	130 μ L

BSM = Basal Salt Medium Solution

SA = 0.05% sodium Azide Solution

TC = Test Chemical solution of 202.81 mg (¹⁴C)copoly VP/ST in 10 mL of THFHG

THF = Tetrahydrofuran

Inoc. = Microbes acclimated to copoly VP/ST

Gluc. = (¹⁴C) glucose

The flask assemblies were placed on a shaker and kept in the dark at 22± 2°C for 28 days. A scintillation vial attached to each flask trapped ¹⁴CO₂ that was released. The vials were replaced and ¹⁴CO₂ was assayed on days 1, 3, 7, 14, 21, and 28. About 30% of the (¹⁴C) glucose in the positive control was metabolized to ¹⁴CO₂. The total ¹⁴CO₂ that was obtained in the polymer test flasks averaged about 0.006%.

On day 28 of the test, the contents of each biometer flask were removed and filtered. The clear filtrate was assayed for radioactivity. The filtered solids were extracted with pyridine, then dried, combusted, and the combustion product (i.e., CO₂) assayed for radioactivity. Finally, the pyridine extract was assayed for radioactivity. Almost all (97% to 98%) of the radioactivity was found in the pyridine extract of the filter paper and solids.

To determine if there was any evidence of primary degradation of (^{14}C) copoly VP/ST by the microorganisms, samples of the pyridine extracts also were assayed using gel permeation chromatography. The chromatograms showed no evidence of (^{14}C) copoly VP/ST biodegradation.²¹

Based on these data, it is reasonable to conclude that copoly VP/ST is quite resistant to biodegradation by wastewater treatment organisms (<0.006%) even if they are acclimated to this polymer.

c. Terrestrial Environment

Biodegradation of Copoly VP/ST in Soil

A soil metabolism test was performed to determine the extent to which copoly VP/ST will biodegrade in the terrestrial environment.²² Three different soil types were collected and used in this study. These soils were identified as sandy loam, loam, and loamy sand. Each of the three soil types were brought to 75% of field moisture capacity and used in the following fashion in this study. Twelve samples of approximately 2.5 g of each soil type were added to 50 mm x 10 mm cellulose extraction thimbles. The samples were divided into four groups of three each by treating with: (1) 50 μL of a test chemical solution of (^{14}C) copoly VP/ST in tetrahydrofuran (THF); (2) 50 μL of THF (negative control); (3) 50 μL of the test chemical solution of (^{14}C) copoly VP/ST in THF to soil which was autoclaved at 121°C (sterile control); or (4) 130 μL of (^{14}C) glucose (positive control). Each thimble was placed in a 50-mL biometer flask. The biometer flask assemblies were placed on a shaker in the dark at $22 \pm 1^\circ\text{C}$ for 28 days. A scintillation vial, containing 1 mL of diethanolamine attached to each flask, trapped the $^{14}\text{CO}_2$ released. The vials were removed and assayed on days 3, 7, 14, 21, and 28 and fresh vials containing diethanolamine were attached on days 3, 7, 14, and 21.

Cumulative measurements of $^{14}\text{CO}_2$ evolution were made. From 54% to 60% of the (^{14}C) glucose in the positive control was metabolized to $^{14}\text{CO}_2$. The total $^{14}\text{CO}_2$ that was released in the polymer test flasks ranged from 0.0015% to 0.0021%.

On day 28, the solids in each biometer flask were extracted with pyridine. About 77% to 89% of the radioactivity was extracted from the soil using pyridine. The pyridine extracted soils were then combusted to attempt to obtain a mass balance for the ^{14}C -labeled compound. About 4% to 18% of the radioactivity was not extracted by

pyridine. To see if there was any evidence of primary degradation of copoly VP/ST in these soils, samples of the pyridine extracts were analyzed using gas permeation chromatography and compared with polystyrene standards. Examination of the chromatographs showed no evidence of copoly VP/ST degradation in this study.²²

From these data it is reasonable to conclude that, irrespective of the soil type, this polymer is very resistant to biodegradation and is not expected to biodegrade in the terrestrial environment.

d. Summary:

Fate of Styrene and 2-Vinylpyridine in the Environment

The 1.31 lb/year of each monomer that is estimated to enter the soil environment is expected to volatilize into the air. Assuming that the monomers are continuously released from a single point source, and utilizing a technique for approximating the impact of area sources¹⁸, the predicted maximum ambient concentrations of styrene and 2-vinylpyridine are 57 ppt and 61 ppt respectively. This methodology predicts the highest possible concentration of monomers in ambient air.

Fate of Copoly VP/ST in the Environment

Copoly VP/ST is a stable polymeric substance not amenable to biodegradation, and therefore, is expected to remain in soils when disposed of to agricultural land as a component of animal excreta used as manure (fertilizer). Taking into account the very low aqueous solubility (88.6 to 185.6 ppb) of this polymer and the high potential to be absorbed by the soil (1.08×10^5), it is expected to have a low mobility through soil. It seems reasonable to assume that copoly VP/ST will not reach surface or ground waters from agricultural runoff from soils to which it has been disposed. As mentioned previously, this is a high molecular weight polymer and no loss due to volatilization is expected. Based on its high molecular weight, low aqueous solubility, and lack of absorption by the rat, this polymer is not expected to bioaccumulate in plants or terrestrial organisms.

8. Environmental Effects of Released Substances

a. 2-Vinylpyridine and styrene

Environmental Effects:

Air

There are no national ambient air standards for styrene and 2-vinylpyridine. In cases where national air standards are not available and a comparison number is needed for risk assessment, one approach is to start with a well accepted occupational health standard such as the American Conference of Industrial Hygienists Threshold Limit Values (TLV's) and divide by appropriate safety factors. The safety factors are intended to account for the following differences:

- 1). Hours per week of exposure - 40 for workforce, 168 for the general population;
- 2). Years per lifetime of exposure - 50 for workforce, 100 for the general population;
- 3). Nature of the population - 18-65 years old and healthy for the workforce versus a variable group for the general population, including the young, the old and infirm;
- 4). The nature of the exposure - voluntary for the workforce versus involuntary for the general population;
- 5). The ability to observe effects - easier in the workforce, harder in the general population; and,
- 6). The size of the population - workforce smaller, general population larger.

Depending on the toxicity of the chemical being considered, different safety factors are used. For example, one ambient air guideline uses a factor of 300 for high and moderate toxicity contaminants and 50 for low toxicity contaminants.²⁵ In the referenced guideline, styrene is considered a moderate toxicity contaminant. Applying a safety factor of 300 to the 1986-7 TLV, an ambient air screening guideline of $50 \text{ ppm}/300 = 0.12 \text{ ppm}$ is obtained. The estimated air level of styrene from release of the monomer to the air for the above application using the worse case scenario is three orders of magnitude below the ambient air screening level.

In cases where a documented occupational health value does not exist, comparison to compounds of similar structure and toxicity for which an occupational value does exist is often used. No occupational health limit is available for 2-vinylpyridine. Using the TLV for pyridine, and using a safety factor of 300 for high toxicity contaminants, an ambient air screening guideline of $5 \text{ ppm}/300 =$

002588

0.017 ppm or 17 ppb is derived. The estimated air level for 2-vinylpyridine from release of the monomer to the air for the above application using the worse case scenario is greater than two orders of magnitude below the ambient air screening level.

Water and Soil:

Summaries of the environmental effects of styrene and 2-vinylpyridine on aquatic life, waste treatment facilities, and plants are given in Tables 1 and 2. The data are taken from Eastman Kodak Company Environmental Safety Data Sheets which are in Appendices 5 and 6.

Table 1

Environmental Effects of Styrene
AQUATIC EFFECTS

Test Species	Test Duration	LC50* Point Estimate
Fathead Minnow <u>Pimephales promelas</u> (Pp)	96 hr	> 100 µL/L (Hard Water)
Fathead Minnow <u>Pimephales promelas</u> (Pp)	96 hr	46.4 µL/L (Soft Water)
Water Flea <u>Daphnia magna</u> (Dm)	96 hr	> 100 µL/L
Sideswimmer <u>Gammarus fasciatus</u> (Gf)	96 hr	>100 µL/L
Snail <u>Helisoma trivolvis</u> (Ht)	96 hr	>100 µL/L
Bluegill Sunfish <u>Lepomis macrochirus</u> (Lm)	96 hr	25.1 µL/L (Soft Water)
Goldfish <u>Carassius auratus</u> (Ca)	96 hr	64.7 µL/L (Soft Water)

*LC50 = Lethal Concentration to 50% of the population.

SECONDARY WASTE TREATMENT COMPATIBILITY

5-hr IC50* = > 5000 µL/L

*IC50 = The concentration of chemical that inhibits, by 50%, glucose metabolism by secondary waste treatment microorganisms.

PLANT GROWTH EFFECTS

GERMINATION	Test Duration	No Observed Effect Concentration (adverse)
Ryegrass (<u>Lolium perenne</u>)	7 Days	100 µL/L
Radish (<u>Raphanus sativus</u>)	7 Days	100 µL/L
Lettuce (<u>Lactuca sativa</u>)	7 Days	100 µL/L

Table 2

Environmental Effects of 2-Vinylpyridine AQUATIC EFFECTS

Test Species	Test Duration	LC50* Point Estimate and (95% Confidence Limits)	No Observed Effect Concentration (Acute)
Fathead Minnow <u>Pimephales promelas</u> (Pp)	96 hr	4.0 µL/L (1-10 µL/L)	1.0 µL/L
Water Flea <u>Daphnia magna</u> (Dm)	96 hr	3.2 µL/L (1-10 µL/L)	1.0 µL/L
Sideswimmer <u>Gammarus fasciatus</u> (Gf)	96 hr	3.2 µL/L (1-10 µL/L)	1.0 µL/L
Flatworm <u>Dugesia tigrina</u> (Dt)	96 hr	3.2 µL/L (1-10 µL/L)	1.0 µL/L
Snail <u>Helisoma trivolvis</u> (Hr)	96 hr	3.2 µL/L (1-10 µL/L)	1.0 µL/L

002590

Segmented Worm <u>Lumbriculus variegatus</u> (Lv)	96 hr	32 µL/L (10-100 µL/L)	10.0 µL/L
Pillbug <u>Asellus intermedius</u> (Ai)	96 hr	3.2 µL/L (1-10 µL/L)	1.0 µL/L

*LC50 - Lethal Concentration to 50% of the population.
Point Estimate Calculated By: Non-Linear Interpolation.

SECONDARY WASTE TREATMENT COMPATIBILITY

5-hr IC50* = 1200 µL/L

*IC50 - The concentration of chemical that inhibits, by 50%, glucose metabolism by secondary waste treatment microorganisms.

PLANT GROWTH EFFECTS

GERMINATION	Test Duration	No Observed Effect Concentration (adverse)
Ryegrass (<u>Lolium perenne</u>)	7 Days	100 µL/L
Radish (<u>Raphanus sativus</u>)	7 Days	100 µL/L
Lettuce (<u>Lactuca sativa</u>)	7 Days	100 µL/L

Human Health Effects

2-Vinylpyridine (2-VP):²⁶

Acute and Short-Term Studies.²⁶ Toxicity studies in animals from the Laboratory of Industrial Medicine, Eastman Kodak Company, and the Russian literature show 2-VP to be fairly acutely toxic and to be readily absorbed through the skin following contact with the liquid or vapor. More extensive acute studies and a 14-day gavage study in rats were performed by the Toxicology Section, Health and Environment Laboratories, Eastman Kodak Company. The oral LD₅₀ of 2-VP was intubated, but when gavaged as a 20% suspension in corn oil the LD₅₀ was 951 mg/kg in fasted and fed male rats, 673 mg/kg in fasted female rats, and 951 mg/kg in fed female rats. The 2-VP was a strong eye irritant to rabbits and strong irritant and sensitizer for guinea pig skin. The dermal LD₅₀ on guinea pigs was 0.16 mL/kg.²⁷

Subchronic Study.²⁸ A 90-day subchronic gavage study in which rats were dosed by gavage with suspensions of 2-VP in corn oil was performed by Toxicology Section, Health and Environment Laboratories, Eastman Kodak Company. doses were 0, 20, 60, and 180 mg/kg per day, 5 days per week, for 13 weeks. Systemic effects at the high dose included: reduced body weight gain in male rats; reduced feed consumption in male rats, and, toward the end of the study, increased feed consumption in female rats; a slight increase in number of platelets in both sexes; a slight increase in aspartate amino transferase (AAT) in male rats; increased or decreased absolute organ weights of brain, heart, and adrenal glands, and relative to body weight, organ weights of liver, absolute organ weights of liver and relative weights of liver, kidneys, and ovaries in females. The middle dose produced increased AAT in male rats, increased relative liver to brain weights in female rats.

The higher doses of 60 to 180 mg/kg also produced local effects due to irritation of the gastric mucosa. These involved primarily the nonglandular epithelium and were characterized by degeneration of the superficial epithelium and were characterized by degeneration of the higher does, hyperkeratosis and acanthosis of the epithelium resulting in thickening of the nonglandular epithelium and mild inflammatory changes (congestion, edma, and inflammatory cell infiltration). No other remarkable gross or microscopic pathology was observed at any dose.

The lowest doe of 20 mg/kg did not produce any significant local or systemic changes in either sex.^{27,29}

Styrene³⁰

An extensive review of the toxic effects of tyrene in man and animals and an evaluation of these effects with regard to setting worker protection standards has been published by the U.S. National Institute of Occupational Safety and Health.³¹

Acute Toxic Effects. Styrene is readily absorbed through the lungs of man and animals from the gaseous phase and, less rapidly, through the skin from the gaseous and liquid phases. Absorbed styrene is readily detoxified and eliminated, partially through the lungs, but mainly through the elimination of metabolites in the urine. The acute oral LD₅₀ of styrene is reported to be 5000 mg/kg in rats and 360 mg/kg in mice. Acute exposure to the liquid or vapor produces mainly irritation of the skin, eyes, and respiratory tract. It affects the central nervous system of animals and man and produces in humans, following prolonged exposures to high levels, neurological disturbances, but has no acute effect on the hematopietic system of animals.

Subchronic and Long-Term Studies. In a series of whole-body exposure studies, guinea pigs exposed to sytrene were susceptible to acute lung infiltration and death at vapor levels that had no effect on rats, rabbits, or dogs. No significant changes were found on gross and microscopic pathology in any animals. Oral intubationof 667 mg/kg of styrene per day in rats produced slight effects on growth and organ weights; 133 mg/kg was a NOEL. Rats exposed to 300 ppm styrene vapor for 11 weeks had liver alterations. Rats dosed in drinking water with 7.7 and 14 mg/kg (males) or 12 and 21 mg/kg (females) of styrene for 2 years showed no significant differences from controls in any physiological value measured, in clinical signs, or in

incidences of tumors or other lesions found on gross or microscopic pathology (Litton).

In dogs, styrene intubated at levels of 400 or 600 mg/kg per day for up to 561 days produced consistent changes in the formed blood elements that were readily reversible when administration of styrene was stopped; 200 ppm was a NOEL. No significant toxicity was seen on gross or microscopic pathology.

Effects on the liver and hematological and pulmonary systems and on the central nervous system have been reported in humans exposed to styrene for extended periods. It is not possible to relate these findings to dose.

Reproductive Effects. Several studies in rats by whole-body exposure at 12 or 47 ppm styrene vapor or by gavage at 90 or 150 mg/kg twice a day indicate that styrene is not embryotoxic, fetotoxic, or teratogenic in rats at levels that are toxic to the dams. Rats and rabbits exposed by whole-body exposure to 300 and 600 ppm styrene vapor showed no embryotoxic, fetotoxic, or teratogenic effects. Pregnant mice exposed to 250 to 1000 ppm of styrene vapor showed increased embryoletality and terata at doses that were toxic to the dams. Hamsters exposed to 750 ppm styrene showed no significant effect on fetuses, whereas at 1000 ppm there were embryoletal effects, but no malformations were found.

Epidemiological studies in humans have given conflicting results, and in many studies confounding factors could not be properly controlled. Several studies, however, have suggested that styrene may adversely affect reproductive success, evidenced as spontaneous abortions in females occupationally exposed to high levels of styrene.

Mutagenic and Clastogenic Effects. Styrene has produced extremely irregular results in in vitro short-term predictive tests. Similar uneven results have been found in experiments in animals and in studies of humans occupationally exposed to styrene. Taken together, the results suggest that styrene is a weak mutagen and clastogen.

Carcinogenesis. Styrene has been studied for oncogenic effects in 8 recent and more or less adequate studies as follows: In rats by inhalation at 600 and 1000 ppm of styrene (Dow); by gavage in olive oil in 2 strains of mice and 1 strain of rats (IARC); a mixture of styrene and beta-nitrostyrene by gavage in rats and mice (NTP); and styrene by gavage in rats and mice (NTP). In the Dow study, while

neither male nor female rats showed a significant increase in tumors of any site, female rats had nonsignificant increased in the combined frequencies of lymphoid and hematopoietic system tumors. In the IARC studies, which were complicated by deaths due to surpassing the MTD, one strain of mice showed a significant increase in liver tumors in females and a nonsignificant increase in males, whereas the other mouse strain and the rats showed only nonsignificant increases in tumor incidence at a few livers sites. Styrene caused significant toxic lesions of the liver in the first mouse study. In the bioassay of a mixture of styrene and beta-nitrostyrene, NTP concluded that there was no convincing evidence that the mixture was carcinogenic in rats or in mice. In the NTP gavage study of styrene, there was a significant positive association between styrene dose and incidence of combined adenomas and carcinomas of the lung of male mice compared to the contemporary control rats. The variation of incidence of these neoplasms in historical control mice in the laboratory during the tests, however, precluded a firm conclusion of carcinogenicity at that site. There were no other significant differences in tumor rates among either species or sex. In addition, no excess tumors were found in the Litton 2-year drinking-water study referred to above.

Epidemiological studies of workers occupationally exposed to styrene have always shown a decrease in total deaths and total deaths from cancer compared to the general population (healthy work phenomenon). This is also true for site-specific cancer incidence except for 2 studies showing an increase in leukemias. The workers in these 2 studies may have been exposed to benzene or other solvents.

In addressing the question of carcinogenicity, NIOSH comments (p. 128), "From the experimental animal investigations and from the epidemiological studies, there seem little basis to conclude that styrene is carcinogenic." They comment further, "If styrene oxide is an intermediate metabolite, covalent binding to nucleic acids leading to cancer development might be predicted; however, there is little evidence that this epoxide is formed in vivo. Nonetheless, the enzyme catalyzing the formation of this epoxide from carbon-carbon double bonds exists in many tissues, as do the enzymes catalyzing the hydration or other inactivation of the epoxide." And conclude, "Thus, while it does not seem appropriate from presently available evidence to conclude that styrene can cause cancer among exposed workers, there is enough evidence to suggest that it might be at least a weak carcinogen, and priority should be given to further studies of this

problem." There has been no recent evidence that would change this position.³⁰

Conclusion

The calculated maximum annual release of 2-vinylpyridine and styrene to the environment is 1.31 lb of each. This is approximately 0.21 ppt of each in soil, much less in water and 60 ppt in air, if they are transferred completely to these compartments. The data in Tables 1 and 2 for soil and water and the threshold limit value arguments for air indicate that these monomers will have virtually no effect on the environment at the maximum use levels. In addition, the mammalian toxicity of these two monomers discussed above is similarly very low compared to the maximum expected concentrations. Therefore, it is concluded that no environmental effects are likely to result from either of these monomers.

b. Copoly VP/ST

Environmental Effects:

The petitioner has examined the effects of copoly VP/ST on plant seedlings, secondary waste treatment microorganisms, and earthworms.

Plant Seedling Effects

A study was performed to determine the maximum concentration of copoly VP/ST which has no effect on the germination and early growth of corn, lettuce, marigold, radish, and soybean³²

Thirty seeds of each of the five-plant species were exposed to 0, 10, 100, and 1000 mg copoly VP/ST per kg of bedding material under controlled conditions in a greenhouse. The study was 21 days long. The percent plant seed germination, height of each seedling, and root length of each seedling were measured at day 21 and compared statistically with control values. Abnormal characteristics were also recorded at the time of harvesting. The statistical analyses used for this study were based on U.S. FDA Guidelines for Statistical Analysis of Plant Growth Data.³³ Statistical techniques used included: descriptive statistics, log 10 transformation of dose groups, nested analysis of variance (ANOVA), Scheffe's Test, Dunnett's Test, a test for linear trend in proportions, power calculations for the ANOVA, and Levene's Test.

002596

There were no statistically significant adverse effects at 1000 mg/kg. Plant height and plant root length in all five species were not significantly affected by any of the doses of copoly VP/ST. The same lack of significant polymer effect was seen from the data on the percent germination of the seeds of these plants. Of the approximately 600 individual plants used in this study, only five were observed to have any abnormal appearance. These were: corn (10 mg/kg dose) - two plants each with a torn leaf and one plant with a chlorotic leaf; corn (control, 0 mg/kg dose) - two plants each with a small leaf tear.³²

Based on the results of this study, terrestrial plants exposed to <1000 mg/copoly VP/ST per kg soil are not expected to be affected adversely.

Secondary Waste Treatment Compatibility

A radiorespirometric method was used to determine the compatibility of copoly VP/ST with secondary waste treatment microorganisms.³⁴

In this study, sludge microorganisms efficient in metabolizing glucose were exposed to several concentrations of copoly VP/ST, the highest of which did not exceed 1/4 of its limiting aqueous solubility.

The ratios of the % conversions of D-(U-¹⁴C) glucose to ¹⁴CO₂ in the presence of various levels of copoly VP/ST to the % conversion of D-(U-¹⁴C) glucose to ¹⁴CO₂ in the absence of copoly VP/ST were used to define a dose-response relationship. Potassium dichromate was used as the positive control.

To each of 27 respirometer flasks was added 5.0 mL of activated sludge solution that contained 10 mg of sludge solids per mL. Four groups of flasks (three replicates per group) then received varying amount of copoly VP/ST. Another four groups of flasks (three replicates per group) received varying amounts of dichromate. The remaining 3 flasks received 2.5 mL of 0.02 M phosphate buffer (negative control). Each of the 27 flasks then received 2.5 mL of a D-(U-¹⁴C) glucose solution (approximately 0.3 μCi). The resulting copoly VP/ST concentrations were 24 μ/L, 2.4 μg/L, 0.24 μg/L, and 0.024 μg/L. The dichromate (positive control) exposure concentrations were 333 mg/L, 167 mg/L, 33 mg/L, and 3.3 mg/L. The flasks were sealed with clean serum stoppers and incubated in the dark at 24°C in a reciprocating incubator-shaker for 5 hours. At the end of the incubation period the contents of each respirometer flask were acidified. The ¹⁴CO₂ absorbing vials were detached from the

respirometers and the trapped $^{14}\text{CO}_2$ assayed in a scintillation spectrometer.

In the negative control samples (without copoly VP/ST or dichromate), 31.46% of the D-(U- ^{14}C) glucose was metabolized to $^{14}\text{CO}_2$. The minimum acceptable value is 15%. the microorganisms showed a concentration-related effect to the positive control (dichromate); this was expected. Copoly VP/ST at all concentrations tested had no effect on glucose metabolism by the microorganisms.³⁴

Copoly VP/ST would not be expected to affect secondary waste treatment microorganisms adversely as a result of exposure to concentrations $<24 \mu\text{g/L}$. This concentration is approximately one-fourth the limiting aqueous solubility of this polymer at pH values similar to those in most soils and surface waters.

Earthworm Toxicity Study³⁵

The petitioner contracted this study to Biospherics Incorporated, Rockville, Maryland. The purpose of the study was to determine the dose-response relationships of copoly VP/ST effects upon earthworms (dungworms).

Dungworms (*Eisenia foetida*) were kept under relatively controlled laboratory conditions and were exposed to soil concentrations of copoly VP/ST of 0, 62.5, 125, 250, 500, or 1,000 mg polymer/kg soil for a 28-day period. Lethal and sublethal effects of copoly VP/ST on this species of earthworm were monitored on days 7, 14, and 28. Significant procedural difficulties in this test prevented a definitive result from being determined; however, it appears accurate to conclude that the following preliminary statements about the toxicity of copoly VP/ST to earthworms can be made.

Mortality in dungworms exposed to graded loses of copoly VP/ST was seen at almost all treatment and control levels at days 7, 14, and 28. the mortality ranged from 0% in the 250 mg/kg dose group to 10% in the 1,000 mg/kg dose group, with 5% mortality in the control group. An LC_{50} appeared to be $>1,000 \text{ mg polymer/kg soil}$. Sublethal effects (soft, soft and flaccid, and moribund) were also monitored. An interpretative description of "soft" and "soft and flaccid" are: "soft" - less muscle tone, but the activity is equivalent to that of a normal worm; and, "soft and flaccid" - soft muscle tone with decreased mucus production and impaired movement when stimulated by prodding. Significant sublethal effects were evident in all treatment groups by

either 7 or 14 days, and became progressively more severe throughout the 28-day experiment. The sublethal characteristic "soft" was observed among all treatments, but "soft and flaccid" was only observed at 250 mg/kg, 500 mg/kg, and 1000 mg/kg, and then only on day 28. "Moribund" effects were not observed in this study.³⁵

Human Health Effects

Except for mutagenicity testing, copoly VP/ST has not been tested for toxicity. However, a polymer having virtually the same molecular weight distribution and chemical and physical properties but prepared from 2-methyl-5-vinylpyridine (2M5VP) has been tested.

Poly (2-methyl-5-vinylpyridine-co-styrene) (copoly 2M5VP/ST) was at most only slightly toxic when given in a single oral dose to rats or mice, having an acute oral LD₅₀ in excess of 3200 mg/kg in each species. When applied to the skin of guinea pigs under an occlusive wrap for 24 hours, it caused only slight edema and some desquamation. No evidence of absorption was apparent. Copoly 2M5VP/ST failed to sensitize any of ten guineas pigs treated. Repeated skin application (ten days) caused only slight and spotty erythema on the five guinea pigs tested. When applied to the eye of rabbits the compound caused slight erythema. Copoly 2M5VP/ST was fed to rats and dogs at up to 5% in the diet for 90 days. There were no effects in either species except for those attributable to decreased feed intake or substitution of a nonnutritive material for part of the diet in high dose rats. Copoly 2M5VP/ST, labeled with carbon-14, was not shown to be absorbed from the gastrointestinal tract of rats. The Center for Veterinary Medicine has agreed that the results of the tests could be extrapolated to the present polymer, copoly VP/ST. Copoly VP/ST was not a mutagen in the standard plate-incorporation salmonella reverse-mutation assay, with and without rat-liver microsomes. These studies demonstrate that copoly VP/ST has very low acute and chronic toxicities in experimental animals and is not absorbed from the gastrointestinal tract. Thus, the polymer is not expected to cause adverse effects to humans during its manufacture, use, or disposal.

c. Summary

Styrene and 2-vinylpyridine are expected to be released in very low amounts (<2 lb per year, nationwide) and toxicity to aquatic and terrestrial organisms is low compared with the expected concentrations (0.21 parts per trillion in soil and less than this value

in air and water). Therefore, it is concluded that these two monomers will have virtually no effect on the environment.

It is assumed that the fatty acids and amino acids are metabolized by the animal and do not reach the environment.

Copoly VP/ST will not have a significant adverse effect on microorganisms or plants at <1000 mg/kg of soil. Significant lethal effects were not observed on earthworms even at the highest level tested, 1000 mg/kg. Responses of worms demonstrated less muscle tone, but with the activity of normal worms, even at the lowest level tested, 62.5 mg/kg. However, at the proposed use levels, disposal of 1.06 mg Copoly VP/ST/kg of soil/year to the environment is not expected to have any adverse effects.

9. Utilization of Natural Resources and Energy

a. Production, Transport, and Use

Copoly VP/ST is produced and transported in France.

The incorporation of the RPAA into dairy cattle feed in the United States is not expected to require any additional energy. The use of electricity or fuel during the feeding of cattle is not expected to require more than would normally be used.

b. Disposal

The polymer portion of the RPAA will be disposed of to the terrestrial environment in the manure (fertilizer). The presence of copoly VP/ST will not require any additional energy requirements.

c. Effects Upon Endangered Species or Historic Places

The petitioner has demonstrated that copoly VP/ST, when used as intended, will not adversely affect plants or animals. Likewise, the disposal of feces containing copoly VP/ST to land is not expected to have any effect on any place or structure identified in the National Register of Historical Places.

d. Disruption of the Physical Environment from the Production of Copoly VP/ST, Use in the Manufacture of RPAA, and Disposition as a Feed Additive to Ruminant Animals

Production of copoly VP/ST and RPAA is through established processes familiar to and used by the petitioner for products other than copoly VP/ST and is expected to have no effect on the local physical environment. Certainly the incorporation of RPAA as produced by Rhone Poulenc for the diets of ruminant animals will have no detectable effect on that environment.

10. Mitigation of Potential Adverse Environmental Effects

Disposal to the terrestrial environment of copoly VP/ST at a maximum annual rate of 1.06 mg/kg as a result of feeding RPAA are not expected to adversely effect plants and animals. Copoly VP/ST has been shown to be resistant to biodegradation in soil and in secondary waste treatment systems; however, is not expected to bioconcentrate in soil or aquatic organisms. Therefore, the environment is not expected to be affected adversely by this proposed use of copoly VP/ST.

11. Alternatives to the Proposed Action

a. Alternatives

As no potential adverse effects have been identified resulting from the proposed action, the petitioner concludes that it is unnecessary to suggest alternative actions.

b. Environmental Benefits of the Proposed Action

It is anticipated that the use of RPAA will increase the efficiency of milk production through more effective use of feed. This increased efficiency of feed utilization could lead to more productive use of the land for raising grain and other agricultural products.

c. Environmental Risks of the Proposed Action

The polymer is not expected to have any adverse effects on wildlife or soil organisms at the concentrations expected to be disposed of to the terrestrial environment (1.06 mg copoly VP/ST per kg soil/year). It is expected to be inert in soil, existing in a finely divided state. The polymer is not expected to affect earthworms, other soil organisms, or plants at anticipated disposal concentrations. The polymer does not have sufficient volatility to present a hazard to the atmosphere. With a limiting solubility of about 90-180 ppb in water, this polymer will not pose a hazard to the aquatic environment.

12. List of Preparers of the Environmental Assessment

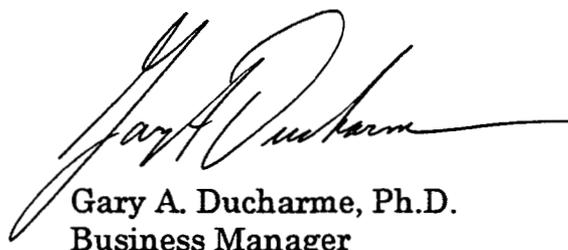
002601

<u>Person</u>	<u>Area of Responsibility</u>
Mr. G. T. Luce Material Safety Program Eastman Chemicals Division Eastman Kodak Company Kingsport, Tennessee	Regulatory Affairs (TSCA, EPA, FDA)
Mr. J. W. Gorsuch Health and Environment Laboratories Eastman Kodak Company Rochester, New York	Environmental Fate and Effects
Dr. E. G. Miller Material Safety Program Eastman Chemicals Division Eastman Kodak Company Kingsport, Tennessee	Regulatory Affairs (FDA)
Mr. Peter Morison Material Safety Program Eastman Chemicals Division Eastman Kodak Company Kingsport, Tennessee	Regulatory Affairs (FDA)
Mr. R. C. Reynolds Health and Environment Laboratories Eastman Kodak Company Rochester, New York	Product Safety and Toxicology
Dr. Kenneth A. Robillard Health and Environment Laboratories Eastman Kodak Company Rochester, New York	Environmental Fate and Effects
Dr. Gary A. Ducharme Rhone Poulenc Animal Nutrition Atlanta, Georgia	Business Manager

13. Certification

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of Rhone Poulenc Animal Nutrition.

002602



Gary A. Ducharme, Ph.D.
Business Manager

14. References

1. Food Additive Petition 2197, Heading H. Enclosure 6, Part A
2. *Idib.*, Part B.
3. CHEMEST: A Program for Chemical Estimation, W. J. Lyman and R. J. Potts, (Editors), Arthur D. Little, Inc., 1985.
4. Eastman Chemicals Division Research Laboratories data, L. A. Cook, 1986.
5. Whittaker, Clark & Daniels, Inc. Material Safety Data Sheet, 1984.
6. The Merck Index, Merck & Co. Inc., Rahway, N.J., 1983, p. 1259.
7. *Ibid.*, p. 858.
8. *Ibid.*, p. 806.
9. Handbook of Environmental Data on Organic chemicals, K. Verschueren, Van Nostrand Reinhold Co., New York, N.Y., 1983, pp. 1055-1056.
10. Reilly Tar and Chemical Corp., Material Safety Data Sheet, 1985.
11. Priv. Comm., Steve Pavlasek, Crop Reporting Board, SRS, USDA, 1986.
12. Eastman chemicals Division Research Laboratories data, L. E. Deetz, 1985.
13. Food Additive Petition 2197, Heading H. Enclosure 1.
14. *Ibid.*, Enclosure 2.
15. Priv. Comm., Charles Strasia, Oklahoma State University Extension Center, to L. E. Deetz, Eastman Kodak Company, 1986.
16. The Feedlot, I. a. Dyer and C. C. Mary, Lea & Febiger, Philadelphia, 1972, pp. 172-173.
17. U.S. FDA, from Sample Environmental Impact Analysis Report provided to Eastman Kodak Company, July 5, 1984.
18. Turner, D.B., Workbook on Atmospheric Dispersion Estimates, EPA Office of Air Programs, P. AP-26. Research Triangle Park, N.C., 1970.
19. C. Tagesson, P.A. Anderson, T. Andersson, T. Bolin, M. Kallberg, and R. Sjudahl, Scand. J. Gastroenterology, 18, 481, 1983).
20. Food Additive Petition 2197, Heading E, Enclosure 15.
21. *Ibid.*, Heading H, Enclosure 6, Part D.
22. *Ibid.*, Part C.
23. *Ibid.*, Heading H, Enclosure 5.
24. *Ibid.*, Enclosure 3.
25. New York State Air Guide -1, Guidelines for the Control of Toxic

002603

- Contaminants, New York State/Department of Environmental Conservation, Division of Air Resources, 1985-6.
26. "NIOSH Recommendations for Occupational Safety and Health Standards", supplement to Morbidity and Mortality Weekly Reports, July 19, 1985, 34, No. 1S, p-26S, HHS Publication No. (CDC) 85-8017, Atlanta, GA.
 27. Food Additive Petition 2197, Heading E, Enclosure 4.
 28. Ibid., Enclosure 5.
 29. Ibid., Enclosures 7, 8 and 9.
 30. Ibid., Enclosure 3.
 31. National Institute for Occupational Safety and Health Criteria for a Recommended Standard: Occupational Exposure to Styrene, U. S. Department of Health and Human Services, Public Health Service, Center for Disease Control, DHHS (NIOSH) Publication No. 83-119, 250pp.
 32. Food Additive Petition 2197, Heading H, Enclosure 6, Part E.
 33. Priv. Comm., Margaret Roper, U.S. FDA, Washington, D.C., 1986.
 34. Food Additive Petition 2197, Heading H, Enclosure 6, Part F.
 35. Ibid., Part G.

15. Appendices

Appendix 1:	Descriptions of Procedures Used to Determine Physical/Chemical Estimates for Copoly VP/ST	36
Appendix 2:	Descriptions of Procedures Used to Determine Physical/Chemical Estimates for Styrene	39
Appendix 3:	Descriptions of Procedures Used to Determine Physical/Chemical Estimates for 2-Vinylpyridine	47
Appendix 4:	Number of Fed Cattle Marketed and Cattle on Fed, 1984-5	55
Appendix 5:	Environmental Safety Data Sheet for Styrene	58
Appendix 6:	Environmental Safety Data Sheet for 2-Vinylpyridine	62

**RHONE POULENC ANIMAL NUTRITION
42, AVENUE ARISTIDE BRIAND
B.P. 100
92164 ANTONY CEDEX, FRANCE**

**RUMEN PROTECTED AMINO ACIDS FOR DAIRY CATTLE
AND DAIRY REPLACEMENT HEIFERS**

JULY, 1994

HEADING H, APPENDIX 1

002605

Appendix 1.

Descriptions of Procedures Used to Determine Physical/Chemical Estimates For Copoly VP/ST

I. CHEMEST Software

A. Introduction

The following estimates - boiling point, melting point, vapor pressure and soil adsorption coefficient - were obtained through the use of CHEMEST software. CHEMEST is an interactive computer system that provides estimates of environmentally important properties of organic chemicals. The procedures within CHEMEST are based on an estimation methods handbook⁽¹⁾ developed by Arthur D. Little, Inc. CHEMEST is available through online services of Technical Database Services, Inc.⁽²⁾

The estimation methods are limited as they are not designed for polymeric materials. The physical/chemical values can be approximated by considering a subunit of the polymer. For copoly VP/ST, an oligomer of 10 monomeric units was chosen. Every CHEMEST output provides information on the method error and, if appropriate, the propagated and total error. Method error for a given estimation method is the error that results when all inputs are accurately known. Propagated error is the additional error in the estimate due to the uncertainty in one or more of the inputs which may be estimated values. Total error is a combination of method and propagated error. It is calculated by CHEMEST whenever one or more of the inputs is an estimate with some non-zero. For additional information on the reported errors see reference (2), Chapter 2.2.7.

B. Vapor Pressure Estimation

The modified Watson method was used to estimate the vapor pressure of the oligomer. The method uses the following equation:

$$\Delta H_v = \Delta H_{vb}(3 - 2(T/T_b))^m$$

where: ΔH_{vb} = heat of vaporization
T = temperature of calculation
 T_b = boiling point
m = constant, calculated from (T/T_b) ratio by
CHEMEST

The heat of vaporization is calculated by CHEMEST using two parameters (a and b) which are associated with the polarity of the

002606

compound. The parameters are determined by choosing the compound class that best represents the polarity of the compound.

The input parameters for the estimation of the vapor pressure for the oligomer included:

Normal Boiling Point	1000.1°C (Estimated)
Melting Point	574.4°C (Estimated)
Identification of Physical State	Solid
Identification of Compound Class	Nonpolar or Slightly Polar Compound (a - 1.0, b - 0.10)
Temperature of Calculation	25.0°C

Since measured boiling point and melting point values were not available for the oligomer, estimates for both of these properties were obtained through CHEMEST. The boiling point was estimated using calculated values for molar refraction and parachor and a correcting parameter for chemical type. CHEMEST determines the molar refraction and parachor by summing fragment values for each atom, multiple bond and/or substructure in the compound. The melting point was estimated using the boiling point as the input parameter.

The estimated vapor pressure for the oligomer was 1.94×10^{-26} mm-Hg with a total error factor of 160.2. The breakdown of this total error factor is as follows:

Method Error:	x 10.0
Propagated Error:	x 92.2
T _b Component:	x 50.8
T _m Component:	x 9.4
Total Error:	x 160.2

CHEMEST calculated a value for vapor pressure but stated a warning message that the value was outside the accuracy range of the method. It can be assumed that the vapor pressure for the polymer would be less than or equal to that for the oligomer.

References: (2), Ch. 14 and (2), Ch. 4-6.

C. Soil Adsorption Coefficient

The soil adsorption coefficient for the oligomer was estimated from the water solubility. The procedure in CHEMEST uses several regression equations which vary by the chemical classes represented.

The following equation was chosen for copoly VP/ST based on the chemical class and low error associated with the correlation: $\log K_{oc} = -0.54 \log S + 0.44$, where S = water solubility (mole fraction).

002607

The chemical classes represented include mostly aromatics or polynuclear aromatics. The input parameter for the estimation was the measured water solubility values for the polymer, 0.18 mg/L at pH 9 (Health and Environment Laboratories Data). ⁽³⁾ The estimated soil adsorption coefficient for the polymer was 1.08×10^5 with a total error factor of 2.0.

Reference: (1), Ch. 4.

II. References

1. Handbook of Chemical Property Estimation Methods, W. J. Lyman, W. F. Reehl, and D. H. Rosenblatt, (Eds.), McGraw-Hill Book Co., 1992.
2. CHEMEST: A Program for Chemical Estimation, W. J. Lyman and R. J. Potts, (Eds.), Arthur D. Little, Inc., 1985.
3. Ziegler, D.A., Specific Buffer Solubility - Poly(2-vinylpyridine-co-styrene) (copoly VP/ST), Health and Environment Laboratories, Report #ETS-TR84-57, 1984.

**RHONE POULENC ANIMAL NUTRITION
42, AVENUE ARISTIDE BRIAND
B.P. 100
92164 ANTONY CEDEX, FRANCE**

**RUMEN PROTECTED AMINO ACIDS FOR DAIRY CATTLE
AND DAIRY REPLACEMENT HEIFERS**

JULY, 1994

HEADING H, APPENDIX 2

002609

Appendix 2.

Description of Procedures Used to Determine Physical/Chemical Estimates for Styrene

I. MedChem Software

A. Introduction

The estimate for the octanol/water partition coefficient (K_{ow}) was obtained through the use of Pomona MedChem Software, Release 3.33.⁽¹⁾ The software is based on a procedure which assigns fragment constants to structural subunits. The calculated $\log K_{ow}$ is the sum of fragment constants appropriate for the molecule in question.⁽²⁾ These calculations have been developed into a computer algorithm called CLOGP.⁽³⁾ The software is a commercially available package from Pomona College.

B. Input Parameter

A chemical nomenclature system, called SMILES, is used to enter the chemical structure as input for the CLOGP algorithm. A SMILES specification uniquely describes the hydrogen suppressed graph of a chemical structure in a way that also defines hydrogen attachments and bond types. More information on SMILES can be obtained from the MedChem Software Manual, Release 3.33. The SMILES input for styrene was:

clcccc(C-C)cl

C. Results

Four types of structural subunits and bonds were defined for the styrene molecule and are listed with their corresponding fragment values in the following table.

<u>Class</u>	<u>Type</u>	<u>Log (P) Contribution</u>	<u>Description</u>	<u>Value</u>
Isolating	Carbon	2	Aliphatic isolating carbon(s)	0.390
Isolating	Carbon	6	Aromatic isolating carbon(s)	0.780
Exfragment	Hydrogen	8	Hydrogen(s) on isolating carbons	1.816
Exfragment	Bonds	1	Chain and 0 alicyclic (net)	-0.120

The CLOGP algorithm calculated the $\log K_{ow}$ value at 2.866. No error level was determined as all fragment constants were measured values.

II. CHEMEST Software

A. Introduction

002610

The following estimates for styrene - boiling point, melting point, vapor pressure, Henry's Law Constant, volatilization from water, water solubility, soil adsorption coefficient and bioconcentration factor - were obtained through use of CHEMEST software. CHEMEST is an interactive computer system that provides estimates of environmentally important properties of organic chemicals. The procedures within CHEMEST are based on an estimation methods handbook⁽⁴⁾ developed by Arthur D. Little, Inc. CHEMEST is available through online services of Technical Database Services, Inc.⁽⁵⁾.

Every CHEMEST output provides information on the method error and, if appropriate, the propagated and total error. Method error, for a given estimation method, is the error that results when all inputs are accurately known. Propagated error is the additional error in the estimate due to the uncertainty in one or more of the inputs which may be estimated values. Total error is a combination of method and propagated error. It is calculated by CHEMEST whenever one or more of the inputs is an estimate with some non-zero. For additional information on the reported errors see reference (5), Chapter 3.2.7.

B. Boiling Point

The Meissner method was used to estimate the normal boiling point of styrene. It is based on correlating the boiling point with molar refraction (R_D), parachor (P), and a parameter for chemical type (B):

$$T_b = \frac{637 (R_D)^{1.47} + B}{(P)}$$

where: T_b = boiling point
 R_D = molar refraction
 P = parachor
 B = constant whose value depends on chemical type

CHEMEST calculates R_D using the atomic refraction contribution method of Eisenlohr which consists of summing contributions for each atom, multiple bond and/or substructure in the compound. Parachor is calculated using a similar contribution scheme. The input values for R_D and P were obtained from Table 12-3 and follow:

<u>Molecular Feature</u>	<u>Number</u>	<u>R_D</u>	<u>P</u>
Benzyl	1	25.207	190.0

002611

Calculated values for R_D and P were 31.8 and 227, respectively. The parameter B is used to adjust the computation of boiling point for compound type. The input value for B was obtained from Table 12-5:

$$B = -2500 \text{ (aromatic hydrocarbon)}$$

The R_D , P and B were input into the above equation to calculate an estimated boiling point for 168°C with 5.0% total error.

Reference: (4), Ch. 12.

C. Melting Point

The following equation was used to estimate the melting point of styrene in the CHEMEST software:

$$T_m = 0.5839 T_b$$

where: T_m = melting point

T_b = boiling point

The input parameter was the measured boiling point, 145°C. The estimated melting point was calculated at 5.2°C with 8.4% total error.

Reference: (5), Ch. 4-9.

D. Vapor Pressure Estimation

The modified Watson method was used to estimate the vapor pressure of styrene. The method uses the following equation:

$$\Delta H_v = \Delta H_{vb} (3 - 2 (T/T_b))^m$$

where: ΔH_{vb} = heat of vaporization

T = temperature of calculation

T_b = boiling point

m = constant, calculated from T/T_b ratio by CHEMEST

($m = 0.19$ for liquids)

The heat of vaporization is calculated by CHEMEST using two parameters (a and b) which are associated with the polarity of the compound. The parameters are determined by choosing the compound class that best represents the polarity of the compound.

The input parameter for the estimation of the vapor pressure of styrene included:

Normal Boiling Point	145°C (measured)
Identification of Physical State	Liquid
Identification of Compound Class	Nonpolar or Slightly Polar Compounds ($a = 1.50$, $b = 0.10$)
Temperature of Calculation	20.0°C

002612

The estimated vapor pressure was 4.5 mm-Hg with a total error factor of 10.0%.

References: (4), Ch. 14 and (5), Ch. 4-6.

E. Henry's Law Constant

Estimation of Henry's Law Constant for styrene was based on use of the vapor pressure - water solubility ratio:

$$H = P_{vp}/S$$

where: H = Henry's Law Constant in atm-m³/mol

P_{vp} = Vapor pressure in atm

S = Water Solubility in mol/m³

The input parameters for styrene included:

P_{vp} = 5.0 mm-Hg at 20°C (measured)

S = 300 mg/L at 20°C (measured)

Henry's Law Constant was estimated at 2.284 x 10⁻³ atm-m³/mol with a total error factor of 1.0.

Reference: (5), Ch. 4-8.

F. Volatilization from Water Estimation

Henry's Law Constant was used to estimate the volatility rate of styrene from water. The procedure in CHEMEST uses Henry's Law Constant to calculate gas and liquid phase mass transfer coefficients and is based on the following equation:

$$C = C_0 e^{-k_v t} = C_0 e^{-k_L t/Z}$$

where: C₀ = initial concentration (g/cm³)

k_v = volatilization rate constant

t = time

K_L = mass transfer coefficient for liquid phase

Z = mean depth of water body

This equation was obtained through integration of the following expression:

$$N = K_L (C - P/H)$$

where: N = Flux (g/cm²oS)

C = Concentration

P = Pressure

H = Henry's Law Constant

A more elaborate derivation of this equation and explanation of the variables can be found in the estimation methods handbook⁽⁴⁾. CHEMEST assigns values to the variables

002613

associated with these expressions by requiring wind velocity, water velocity, and depth of water body for the calculation.

The input parameters for the estimation of the volatility rate of styrene were:

Temperature of Calculation	20°C
Henry's Law Constant	2.284 x 10 ³ atm-m ³ /mol (estimated)
Wind Velocity	0.10 m/s
Water Current Velocity	0.10 m/s
Depth of Water Body	0.10 m

Resistance to volatilization associated with transfer of the chemical from the bulk of the water to the interface and associated with presence of surface active agents on water surface were not considered.

An estimated volatilization rate constant of 0.379 hour⁻¹ was calculated for styrene with a total error factor of 5.0.

References: (4), Ch. 15 and (5), Ch 4-7.

G. Solubility in Water

The water solubility of styrene was estimated at 25°C using the octanol/water partition coefficient. The procedure in CHEMEST uses 3 different equations depending on the acidity of the compound and the range of log K_{ow} values. These equations are obtained from those recommended by Lyman⁽⁶⁾ and are not listed in the estimation methods handbook⁽⁴⁾ nor the CHEMEST manual⁽⁵⁾.

The input parameters for estimation of the water solubility of styrene were:

Log K _{ow}	2.95 (measured)
Presence of acid group	No

Equation 12 from Lyman's reference ⁽⁶⁾ was used to estimate the water solubility of styrene at 246 mg/L with a method error factor of 1.6.

Reference: (5), Ch. 4-1

H. Soil Adsorption Coefficient

The soil adsorption coefficient for styrene was estimated from the octanol/water partition coefficient. The procedure in CHEMEST

002614

uses several regression equations which vary by the chemical classes represented.

The following equation was chosen for styrene based on the chemical class and low error associated with the correlation:

$$\log K_{OC} = 0.937 \log K_{OW} - 0.006$$

The chemical classes represented include aromatics, polynuclear aromatics, triazines, and dinitroaniline herbicides.

The input parameter for the estimation was the measured $\log K_{OW}$ value, 2.95. The estimated soil adsorption coefficient was 573 with a total error factor of 1.6.

Reference: (4), Ch. 4.

I. Bioconcentration Factor Estimation

The bioconcentration factor for styrene was estimated from the octanol/water partition coefficient. CHEMEST used the following regression equation:

$$\log BCF = 0.76 \log K_{OW} - 0.23$$

The input parameter was the measured $\log K_{OW}$ value, 2.95. The estimated bioconcentration factor was 98 with a total error factor of 3.0.

Reference: (4), Ch. 5.

III. Summary of Physical/Chemical Estimates of Styrene

<u>Physical/Chemical Property</u>	<u>Estimated Value</u>	<u>Measured Value</u>
Octanol/Water Partition Coefficient (Log ₁₀)	2.87 a	(2.95) b
Boiling Point	168°C c	(145.2°C) e (145-146°C) f
Melting Point	5.2°C c	(- 30.63°C) e (- 31°C) f
Vapor Pressure	4.5 mm-Hg c at 20°C	(5 mm Hg d at 20°C)
Solubility in Water	246 mg/L c at 25°C	(300 mg/L d at 20°C)
Henry's Law Constant	2.28 x 10 ⁻¹⁰ atm-m ³ /mol c	
Rate of Volatilization From Water	0.38 hour ⁻¹ c	
Soil Adsorption Coefficient	573 c	
Bioconcentration Factor	98 c	

a- MedChem Software Release 3.33, Medicinal Chemistry Project, Pomona College, Claremont, California, 1985.

b- Pomona College, Medicinal Chemistry Project, "Chemical Parameter Data Base," Leo, A.J. and Hansch, C., (Eds.), Seaver Chemistry Laboratory, Claremont, California, July 1, 1977.

c- CHEMEST: A Program for Chemical Estimation, W. J. Lyman and R. J. Potts, (Eds.), Arthur D. Little, Inc. 1985.

d- Verschueren, K., "Handbook of Environmental Data on Organic Chemicals," Van Nostrand Reinhold Company, New York, N.Y., 1977, 659 pp.

002616

- e- Weast, R.C., (Ed.), "Handbook of Chemistry and Physics," 57th ed., CRC Press, Cleveland, Ohio, 1976.
- f- Health and Environment Laboratories Data, Lab Method No. 1097, 1980.

IV.

References

1. "MedChem Software Manual, Release 3.33", Medicinal Chemistry Project, Pomona College, Claremont, California, April 1985.
2. Hansch, C., and Leo, A., Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley Interscience, New York (1979).
3. Chou, J., and Jurs, P., J. Chem. Inf. Comput. Sci., 19, 172 (1979).
4. Handbook of Chemical Property Estimation Methods, W. J. Lyman and W. F. Reehl and D. H. Rosenblatt (Eds.), McGraw-Hill Book Co., 1982.
5. CHEMEST: A Program for Chemical Estimation, W. J. Lyman and R. J. Potts, (Eds.), Arthur D. Little, Inc., 1985.
6. Lyman, W. J. (Arthur D. Little, Inc.), "Enhancements to CHEMEST Program: Water Solubility,: interim report on Task 67 of EPA Contract No. 68-01-6271, U.S., Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. (October, 1983).

002617

**RHONE POULENC ANIMAL NUTRITION
42, AVENUE ARISTIDE BRIAND
B.P. 100
92164 ANTONY CEDEX, FRANCE**

**RUMEN PROTECTED AMINO ACIDS FOR DAIRY CATTLE
AND DAIRY REPLACEMENT HEIFERS**

JULY, 1994

HEADING H, APPENDIX 3

002618

Appendix 3

Description of Procedures Used to Determine Physical/Chemical Estimates for 2-Vinylpyridine

I. MedChem Software

A. Introduction

The estimate for the octanol/water partition coefficient (K_{ow}) was obtained through the use of Pomona MedChem Software, Release 3.33.(1) The software is based on a procedure which assigns fragment constants to structural subunits. The calculated $\log K_{ow}$ is the sum of fragment constants appropriate for the molecule in question. (2) These calculations have been developed into a computer algorithm called CLOGP.(3) The software is a commercially available package from Pomona College.

B. Input Parameter

A chemical nomenclature system, called SMILES, is used to enter the chemical structure as input for the CLOGP algorithm. A SMILES specification uniquely describes the hydrogen suppressed graph of a chemical structure in a way that also defines hydrogen attachments and bond types. More information on SMILES can be obtained from the MedChem Software Manual, Release 3.33. The SMILES input for 2-vinylpyridine was:

clccnc(C=C)cl

C. Results

Five types of structural subunits and bonds were defined for the 2-vinylpyridine molecule and are listed with their corresponding fragment values in the following table.

<u>Class</u>	<u>Type</u>	<u>Log (P) Contribution</u>	<u>Description</u>	<u>Value</u>
Fragment	#1		Aromatic nitrogen (TYPE 2)	-1.120
Isolating	Carbon	2	Aliphatic isolating carbon(s)	0.390
Isolating	Carbon	5	Aromatic isolating carbon(s)	0.650
Exfragment	Hydrogen	7	Hydrogen(s) on isolating carbons	1.589
Exfragment	Bonds	1	Chain and 0 alicyclic (net)	-0.120

The CLOGP algorithm calculated the $\log K_{ow}$ value at 1.389. No error level was determined as all fragment constants were measured values.

002619

II. CHEMEST Software

A. Introduction

The following estimates for 2-vinylpyridine - boiling point, melting point, vapor pressure, Henry's Law Constant, volatilization from water, water solubility, soil adsorption coefficient and bioconcentration factor - were obtained through use of CHEMEST software. CHEMEST is an interactive computer system that provides estimates of environmentally important properties of organic chemicals. The procedures within CHEMEST are based on an estimation methods handbook⁽⁴⁾ developed by Arthur D. Little, Inc. CHEMEST is available through online services of Technical Database Services, Inc.⁽⁵⁾

Every CHEMEST output provides information on the method error and, if appropriate, the propagated and total error. Method error, for a given estimation method, is the error that results when all inputs are accurately known. Propagated error is the additional error in the estimate due to the uncertainty in one or more of the inputs which may be estimated values. Total error is a combination of method and propagated error. It is calculated by CHEMEST whenever one or more of the inputs is an estimate with some non-zero. For additional information on the reported errors see reference (5), Chapter 3.2.7.

B. Boiling Point

The Meissner method was used to estimate the normal boiling point of 2-vinylpyridine. It is based on correlating the boiling point with molar refraction (R_D), parachor (P), and a parameter for chemical type (B):

$$T_b = \frac{637 (R_D)^{1.47} + B}{(P)}$$

where: T_b = boiling point
 R_D = molar refraction
 P = parachor
 B = constant whose value depends on chemical type

CHEMEST calculates R_D using the atomic refraction contribution method of Eisenlohr which consists of summing contributions for each atom, multiple bond and/or substructure in the compound. Parachor is calculated using a similar contribution scheme. The input values for R_D and P were obtained from Table 12-3 and follow:

002620

<u>Molecular Feature</u>	<u>Number</u>	<u>R_D</u>	<u>P</u>
6 member ring 	1	0	0.8
-C=C-	3	6.569	37.4
Double bond	1	1.733	19.0
C (singly bound)	1	2.418	9.2
N (in ring)	1	2.840	17.5

Calculated values for R_D and P were 26.7 and 159, respectively. The parameter B is used to adjust the computation of boiling point for compound type. The input value for B was obtained from Table 12-5:

$$B = -3000 \text{ (tertiary amine)}$$

The R_D, P, and B were input into the above equation to calculate an estimated boiling point of 210°C with 5.0% total error.

Reference: (4), Ch. 12.

C. Melting Point

The following equation was used to estimate the melting point of 2-vinylpyridine in the CHEMEST software:

$$T_m = 0.5839 T_b$$

where: T_m = melting point
T_b - boiling point

The input parameter was the measured boiling point, 160°C. The estimated melting point was calculated at 15.2°C with 8.4% total error.

Reference (5), Ch. 4-9

D. Vapor Pressure Estimation

The modified Watson method was used to estimate the vapor pressure of 2-vinylpyridine. The method uses the following equation:

$$\Delta H_v = \Delta H_{vb} (3 - 2 (T/T_b))^m$$

where: ΔH_{vb} = heat of vaporization
T = temperature of calculation
T_b = boiling point
m = constant, calculated from T/T_b ratio by CHEMEST

002621

The heat of vaporization is calculated by CHEMEST using two parameters (a and b) which are associated with the polarity of the compound. The parameters are determined by choosing the compound class that best represents the polarity of the compound.

The input parameter for the estimation of the vapor pressure of 2-vinylpyridine included:

Normal Boiling Point	160°C (measured)
Identification of Physical State	Liquid
Identification of Compound Class	Nonpolar or Slightly Polar Compounds (a = 1.50, b = 0.10)
Temperature of Calculation	25°C

The estimated vapor pressure was 3.7 mm-Hg with a total error factor of 2.0.

References: (4), Ch. 14 and (5), Ch. 4-6

E. Henry's Law Constant

Estimation of Henry's Law Constant for 2-vinylpyridine was based on use of the vapor pressure - water solubility ratio:

$$H = P_{vp}/S$$

where: H = Henry's Law Constant in atm-m³/mol

P_{vp} = Vapor pressure in atm

S = Water Solubility in mol/m³

The input parameters for 2-vinylpyridine included:

$$P_{vp} = 3.7 \text{ mm-Hg at } 25^\circ\text{C (estimated)}$$

$$S = 2.75 \times 10^4 \text{ mg/L at } 20^\circ\text{C (measured)}$$

Henry's Law Constant was estimated at 5.119×10^{-5} atm-m³/mol with a total error factor of 2.0.

Reference: (5), Ch. 4-8.

F. Volatilization from Water Estimation

Henry's Law Constant was used to estimate the volatility rate of 2-vinylpyridine from water. The procedure in CHEMEST uses Henry's Law Constant to calculate gas and liquid phase mass transfer coefficients and is based on the following equation:

$$C = C_0 e^{-k_v t} = C_0 e^{-K_L t/Z}$$

where: C₀ = initial concentration (g/cm³)

k_v = volatilization rate constant

002622

t = time
K_L = mass transfer coefficient for liquid phase
Z = mean depth of water body

This equation was obtained through integration of the following expression:

$$N = K_L (C - P/H)$$
where: N = Flux (g/cm²oS)
C = Concentration
P = Pressure
H = Henry's Law Constant

A more elaborate deviation of this equation and explanation of the variables can be found in the estimation methods handbook⁽⁴⁾. CHEMEST assigns values to the variables associated with these expressions by requiring wind velocity, water velocity, and depth of water body for the calculation.

The input parameters for the estimation of the volatility rate of 2-vinylpyridine were:

Temperature of Calculation	25°C
Henry's Law Constant	5.12 x 10 ⁻⁵ atm-m ³ /mol (estimated)
Wind Velocity	0.10 m/s
Water Current Velocity	0.10 m/s
Depth of Water Body	0.10 m

Resistance to volatilization associated with transfer of the chemical from the bulk of the water to the interface and associated with presence of surface active agents on water surface were not considered.

An estimated volatilization rate constant of 0.01910 hour⁻¹ was calculated for 2-vinylpyridine with a total error factor of 5.7.

References: (4), Ch. 15 and (5), Ch. 4-7.

G. Solubility in Water

The water solubility of 2-vinylpyridine was estimated at 25°C using the octanol/water partition coefficient. The procedure in CHEMEST uses 3 different equations depending on the acidity of the compound and the range of log K_{ow} values. These equations are obtained from those recommended by Lyman⁽⁶⁾ and are not listed in the estimation methods handbook⁽⁴⁾ nor the CHEMEST manual.⁽⁵⁾

002623

The input parameters for estimation of the water solubility of 2-vinylpyridine were:

Log K_{ow}	1.80 (measured)
Presence of acid group	No

Equation 12 from Lyman's reference⁽⁶⁾ was used to estimate the water solubility of 2-vinylpyridine at 4.86×10^3 mg/L with a total error factor of 1.6.

Reference: (5), Ch. 4-1

H. Soil Adsorption Coefficient

The soil adsorption coefficient for 2-vinylpyridine was estimated from the octanol/water partition coefficient. The procedure in CHEMEST uses several regression equations which vary by the chemical classes represented.

The following equation was chosen for 2-vinylpyridine based on the chemical class and low error associated with the correlation:

$$\log K_{oc} = 1.029 \log K_{ow} - 0.18$$

The chemical classes represented include a variety of insecticides, herbicides, and fungicides.

The input parameter for the estimation was the measured log K_{ow} value, 1.80. the estimated soil adsorption coefficient was 47 with a total error factor of 1.6.

Reference: (4), Ch. 4.

I. Bioconcentration Factor Estimation

The bioconcentration factor for 2-vinylpyridine was estimated from the octanol/water partition coefficient. CHEMEST used the following regression equation:

$$\log BCF = 0.76 \log K_{ow} - 0.23$$

The input parameter was the measured log K_{ow} value, 1.80. the estimated bioconcentration factor was 13 with a total error factor of 3.0.

Reference: (4), Ch. 5.

III. Summary of Physical/Chemical Estimates of 2-Vinylpyridine

<u>Physical/Chemical Property</u>	<u>Estimated Value</u>	<u>Measured Value</u>
Octanol/Water Partition Coefficient (Log ₁₀)	1.40 ^a	(1.80) ^b
Boiling Point	210°C ^c	(160°C) ^d
Melting Point	15.2°C ^c	
Vapor Pressure	3.7 mm-Hg ^c at 25°C	(10 mm-Hg at 44.5°C) ^e
Solubility in Water	4.86 x 10 ³ mg/L ^c	(2.75 x 10 ⁴ mg/L) ^e
Henry's Law Constant	5.119 x 10 ⁻⁵ atm-m ³ /mol ^c	
Rate of Volatilization From Water	0.0191 hour ⁻¹ ^c	
Soil Adsorption Coefficient	47 ^c	
Bioconcentration Factor	13 ^c	

a- MedChem Software Release 3.33, Medicinal Chemistry Project, Pomona College, Claremont, California, 1985.

b- Health and Environment Laboratories Data, Lab Method No. 1988, (Value determined HPLC in a method similar to ASTM), 1980.

c- CHEMEST: A Program for Chemical Estimation, W. J. Lyman and R. J. Potts, (Eds.), Arthur D. Little, Inc., 1985.

d- Weast, R. C. and M. J. Astle, (Eds.), Handbook of Chemistry and Physics, 63rd Edition, 1982-1983, p. C-488.

e- Material Safety Data. Reilly Tar & Chemical Corporation, 1510 Market Square Center, 151 North Delaware Street, Indianapolis, Indiana, 3/28/85.

002825

IV.

References

1. "MedChem Software Manual, Release 3.33", Medicinal Chemistry Project, Pomona College, Claremont, California, April 1985.
2. Hansch, C. and Leo, A., Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley Interscience, New York (1979).
3. Chou, J. and Jurs, P., J. Chem. Inf. Comput. Sci., 19, 172 (1979).
4. Handbook of Chemical Property Estimation Methods, W.J. Lyman, W. F. Reehl and D.H. Rosenblatt (Eds.), McGraw-Hill Book Co., 1982.
5. CHEMEST: A Program for Chemical Estimation, W. J. Lyman and R. J. Potts, (Eds.), Arthur D. Little, Inc., 1985.
6. Lyman, W.J. (Arthur D. Little, Inc.), "Enhancements to CHEMEST Program: Water Solubility," interim report on Task 67 of EPA Contract No. 68-01-6271, U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. (October, 1983).

002626

**RHONE POULENC ANIMAL NUTRITION
42, AVENUE ARISTIDE BRIAND
B.P. 100
92164 ANTONY CEDEX, FRANCE**

**RUMEN PROTECTED AMINO ACIDS FOR DAIRY CATTLE
AND DAIRY REPLACEMENT HEIFERS**

JULY, 1994

HEADING H, APPENDIX 4

002627

HEALTH, SAFETY, AND HUMAN FACTORS LABORATORY

Environmental Safety Data Sheet

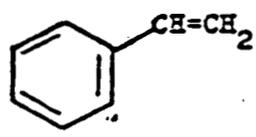
AS Reg. No. 100-42-5 HSHFL No. 77-312 Date: 2/ 24/ 78

Empirical Formula C8H8 Molecular Wt. 104.16

Compound Name Styrene; Vinylbenzene, Ethenylbenzene

Synonyms Phenylethylene, Phenethylene, Phenylethene, Cinnamene

Structure:



TSCA Inventory List: / yes no

Regulatory Status: 1) Currently being tested by NCI for carcinogenicity by Standard Bioassay Protocol as of April 1976. 2) EPA: Selected for Priority Attention as Point Source Effluent Discharge Toxic Pollutant. 3) Among the chemical substances listed in the 19 categories being reviewed for priority testing by the TSCA Interagency Testing Committee for its second-round-of-testing recommendations to the U.S. Environmental Protection Agency.

Environmental Safety Evaluation

I. Physio-Chemical Properties - Tier "O" Data

- () Melting Point -30.63°C, -31°C Solubility-Acetone Soluble, >10^-8
() Boiling Point 145.2°C, 145-146°C at 760 mm Solubility-3A Alcohol Soluble
() Vapor Pressure 10 mm @ 30.8°C Solubility-Octanol >0.1%; >1.0%
() Solubility-H2O 300 mg/l @ 20°C Solubility-Corn Oil >10^-8
() pH of 0.1% aqueous solution pKa-1 Ka

II. Environmental Stability

- () THOD 3.07g/g BOD5 0.55-1.95g/g; 2.45g/g (adapted slw)
() COD 2.80-2.88g/g >1.7g/ml BOD5/COD 0.19-0.85
() BOD20 87% bio. ox.

T 1/2 - Biological (Activated Sludge T.O.C.)

NCI Carcinogenesis bioassay completed as of August 1977.

Solubility-H2O: 280 mg/l at 15°C; 400 mg/l at 40°C. Incomplete Digestion.

002628

() II. Environmental Stability (cont.)

() Products of Biodegradation or Biotransformation:

() T 1/2 - Photochemical _____

() Products of Photodegradation:

() III. Aquatic Toxicity

() LC₅₀ (24, 48, 96h) - Fathead - (Soft water) 56.7, 53.6, 46.4 ppm; (Hardwater) 62.8, 62.59.3 ppm^{5,6}

() LC₅₀ - Daphnia magna _____

() LC₅₀ - Bluegill (soft water) 75.1 (Lepomis macrochirus)^{5,6}

() LC₅₀ - Goldfish (soft water) 64.7 (Carassius auratus)⁵

() LC₅₀ - Guppy (soft water) 74.8 (Gambusia variegatus)⁵

() LC₅₀ - Fathead minnow, Scud, Snails All >100 µl/L⁸

() Special treatments: _____

() IV. Secondary Waste Treatment Compatibility

() IC₅₀ >5,000 mg/L⁸ Other _____

002629

V. Bioconcentration Potential

- () WLN 1 ULR
- () Octanol/water distribution coeff. P=890, log P=2.95⁷
- () Bioconcentration Factor _____
- () T 1/2 - aquatic vertebrate _____

VI. Phytotoxicity - No effect level

- () Selenastrum sp. _____ () Seedling - Corn _____
- () Germination - Ryegrass 100 uL/L () Seedling - Marigold _____
- () Germination - Radish 100 uL/L () Seedling - Radish _____
- () Germination - Lettuce 100 uL/L () Seedling - Lettuce _____

VII. Comments

() VIII. References: See attached page.

() IX. Environmental Hazard - Tier Testing Ratings (See: HS/HF Laboratory Report, "A Tier Testing Scheme," October, 1977)

	Low (1)	Med. (2)	High (3)
() Persistence	1		
() Incompatibility	1		
() Toxicity		2	
() Accumulation		2	

Prepared by:

Philip W. Jenkins, Ph.D.
February 24, 1978

Updated by:

Joseph W. Gorsuch
August 26, 1981

References for the Environmental Safety Data Sheet on
Styrene; CAS Reg. No. 100-42-5.

1. Christensen, H.E., Ed., "Suspected Carcinogens, 2nd Edition. A Subfile of the NIOSH Registry of Toxic Effects of Chemical Substances," National Institute for Occupational Safety and Health, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio, December, 1976.
2. Fairchild, E.J., Ed., "Registry of Toxic Effects of Chemical Substances—1978 Edition," National Institute for Occupational Safety and Health, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio, October, 1978.
3. Bureau of National Affairs, Inc., "ITC Looks at Chemicals Recommended for Inclusion in Next Set of Dossiers," Chemical Regulation Reporter, 1(45), 1575, 1594-7 (January 20, 1978).
4. Weast, R.C., Ed., "Handbook of Chemistry and Physics," 57th ed., CRC Press, Cleveland, Ohio, 1976.
5. Verschueren, K., "Handbook of Environmental Data on Organic Chemicals," Van Nostrand Reinhold Company, New York, N.Y., 1977, 659 pp.
6. Hann, R.W., Jr. and Jensen, P.A., "Water Quality Characteristics of Hazardous Materials," Environmental Engineering Division, Civil Engineering Department, Texas A&M University.
7. Pomona College, Medicinal Chemistry Project, "Chemical Parameter Data Base," Leo, A.J. and Hansch, C., Eds., Seaver Chemistry Laboratory, Claremont, California, July 1, 1977.
8. Health, Safety, and Human Factors Laboratories Data, Lab. No. 77-312. Environmental testing was completed in March, 1980.

**RHONE POULENC ANIMAL NUTRITION
42, AVENUE ARISTIDE BRIAND
B.P. 100
92164 ANTONY CEDEX, FRANCE**

**RUMEN PROTECTED AMINO ACIDS FOR DAIRY CATTLE
AND DAIRY REPLACEMENT HEIFERS**

JULY, 1994

HEADING H, APPENDIX 5

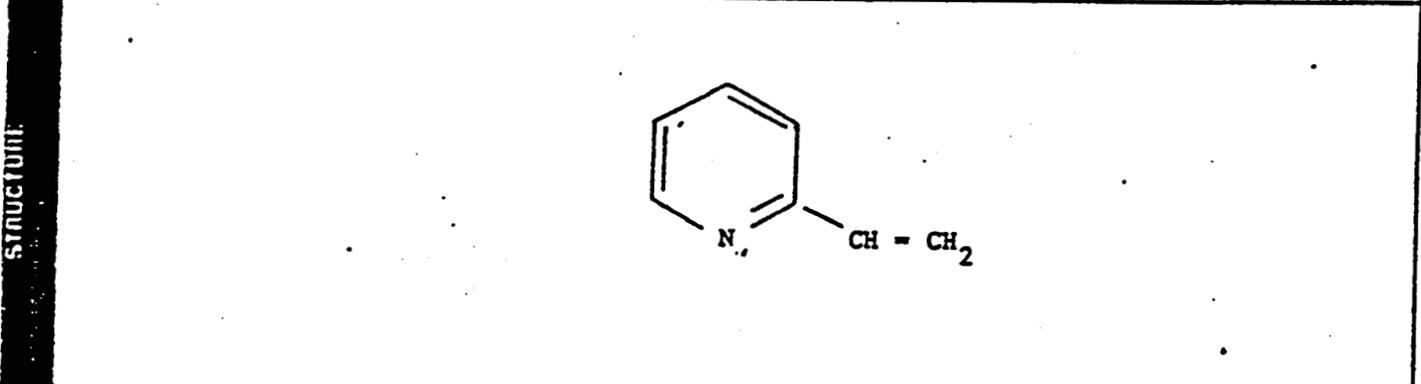
002632

TEST NO.	HAEL NO. 83-0149	SRID NO. Reilly Lot 30912	ACCESSION NO. 905464
----------	---------------------	------------------------------	-------------------------

COMPOUND NAME
 2-Vinylpyridine

SYNONYMS

MOLECULAR FORMULA C ₇ H ₇ N	MOLECULAR WT.
--	---------------



EVALUATION	ENVIRONMENTAL FATE	E-ESTIMATE	LOW (1)	MEDIUM (2)	HIGH (3)
		PERSISTENCE			3
	ENVIRONMENTAL EFFECTS	BIOCONCENTRATION	1		
		ACUTE AQUATIC EFFECTS			3
		SECONDARY WASTE TREATMENT EFFECTS		2	
		PLANT EFFECTS		2	

CHEMICAL PROPERTIES	H ₂ O SOLUBILITY	>1.0% <10%	OCTANOL	>10%	TOD	2.10 g/g
	PK _a		K _a		COD	0.742 g/g
	MELTING POINT		BOILING POINT		BOD ₅	No measurable BOD
	VAPOR PRESSURE				BOD ₂₀	No measurable BOD

REMARKS The structure was confirmed by IR. The purity was reported as 98.95%, by GC analysis.

BIOCONCENTRATION log P = 1.8.
 The log P was estimated by HPLC analysis.

BIODEGRADATION A 21-day biodegradation test utilizing acclimated sludge microorganisms as the inoculum showed 3.29% degradation of the test article as measured by carbon dioxide evolution.

PHOTODEGRADATION

CHEMICAL DEGRADATION

IAEL NO.

83-0149

ENVIRONMENTAL SAFETY DATA SHEET
 HEALTH AND ENVIRONMENT LABORATORIES

ENVIRONMENTAL SCIENCES SECTION

ACCESSION NO.

905464

AQUATIC EFFECTS

LC ₅₀ - <u>PIMEPHALES PROMELAS</u> (FATHEAD MINNOW)	4.0uL/L ^A (1-10uL/L) ^B	LC ₅₀ - <u>MELISOMA TRIVOLVIS</u> (SNAIL)	3.2uL/L ^A (1-10uL/L) ^B
LC ₅₀ - <u>DAPHNIA MAGNA</u> (WATER FLEA)	3.2uL/L ^A (1-10uL/L) ^B	LC ₅₀ - <u>LUMBRICULUS VARIEGATUS</u> (SEGMENTED WORM)	3.2uL/L ^A (10-100uL/L) ^B
LC ₅₀ - <u>GAMMARUS FASCIATUS</u> (SIDESWIMMER)	3.2uL/L ^A (1-10uL/L) ^B	LC ₅₀ - <u>APELLUS INTERMEDIUS</u> (PILLBUG)	3.2uL/L ^A (1-10uL/L) ^B
LC ₅₀ - <u>OUGESIA TIGRINA</u> (FLATWORM)	3.2uL/L ^A (1-10uL/L) ^B	LC ₅₀ - OTHER	

REMARKS
 A = nonlinear interpolated 96-hr LC₅₀.
 B = the LC₅₀ range within the 95% confidence limits.

SECONDARY WASTE TREATMENT COMPATIBILITY

IC₅₀ 1200 mg/L REMARKS

PLANT GROWTH EFFECTS
NO EFFECT CONCENTRATION

GERMINATION	<u>LOLIUM PERENNE</u> (RYEGRASS)	10 uL/L	SEEDLING	<u>ZEA MAYS</u> (CORN)
	<u>RAPHANUS SATIVUS</u> (RADISH)	10 uL/L		<u>TAGETES PATULA</u> (MARIGOLD)
	<u>LACTUCA SATIVA</u> (LETTUCE)	10 uL/L		<u>LACTUCA SATIVA</u> (LETTUCE)
	OTHER			<u>RAPHANUS SATIVUS</u> (RADISH)

REMARKS

REFERENCES COMMENTS

1) "Sequential Testing for Chemical Risk Assessment",
Environmental Risk Analysis For Chemicals, Van Nostrand Company, 1982, pp. 412-433.

PREPARED BY

Joseph W. Gorsuch

DATE

November 14, 1986

002034

**RHONE POULENC ANIMAL NUTRITION
42, AVENUE ARISTIDE BRIAND
B.P. 100
92164 ANTONY CEDEX, FRANCE**

**RUMEN PROTECTED AMINO ACIDS FOR DAIRY CATTLE
AND DAIRY REPLACEMENT HEIFERS**

JULY, 1994

HEADING H, APPENDIX 6

002635

FINDING OF NO SIGNIFICANT IMPACT

for
RUMEN PROTECTED AMINO ACID

for
BEEF CATTLE

Food Additive Petition Number 2197

Eastman Kodak Company
Eastman Chemical Division
Kingsport, Tennessee

The Center for Veterinary Medicine has carefully considered the potential environmental impact of this action and has concluded that this action is not expected to have a significant impact on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

Eastman Kodak Company, Kingsport, Tennessee is requesting the approval of FAP 2297 for the use of rumen protected amino acids (RPAA) for beef cattle in feedlots and farmer-feeder operations. RPAA is in a form that will protect the amino acids (methionine and lysine) from destruction in the rumen and release the nutrients for absorption. The protecting component of the RPAA (copoly VP/ST) is then released unchanged into the environment. In support of this requested approval, Kodak has provided the attached May 14, 1990, environmental assessment (EA).

The EA was placed on display in the Food and Drug Administration Dockets Management Branch for 30 days and its availability was advertised in the October 31, 1991, FEDERAL REGISTER Notice. The comment period closed on December 2, 1991. No comments were received.

Information in the EA indicates that RPAA consists of lysine, methionine stearic acid, talc and copoly VP/ST. Copoly VP/ST is produced at Arkansas Eastman Company, Batesville, Arkansas. The final RPAA product is produced at Tennessee Eastman Company in Kingsport, Tennessee. Information in the EA indicates that employees at these facilities are provided with the necessary safety information and that the facilities are in compliance with the appropriate Federal, State and local environmental requirements. Therefore, no significant impacts to the environment are expected at the sites of manufacture.

Information in the EA indicates that three of the five components of RPAA, lysine, methionine and talc, are naturally occurring products. A fourth component, stearic acid, is a edible fatty acid. Because of the nature of the compounds, the EA only addresses them to a limited, but sufficient, extent to

002636

determine that they will not contribute to the potential for the product to have a significant impact in the environment. The component most prominently covered in the EA is the copoly VP/ST.

Copoly VP/ST is expected to enter the environment through the manure at appreciable levels. Although the EA indicates that the amount that is expected to enter the environment is 1.17 ppm, the correct calculated value is 1.13 ppm. To address the potential for copoly VP/ST to have impact in the environment, Kodak undertook numerous studies to estimate its environmental fate and potential effects. The data in the EA indicate that copoly VP/ST will enter terrestrial environment through manure and that it will subsequently bind to soils. This binding is expected to eliminate the potential for copoly VP/ST to move into aquatic environment. Data in the EA also indicate that no degradation through physical or biological means are expected to occur. Therefore, effects data were determined for terrestrial species. In particular, Kodak has conducted two seedling growth tests and a dungworm toxicity test with copoly VP/ST.

The first seedling growth test found statistically significant adverse dose related trends in corn and soybean plant height and lettuce root length. Although a trend was reported, no significant effects in individual parameters when compared to controls were observed, even at 1000 ppm. However, because of the trend and questions concerning the biological significance of the trend, a follow-up seedling growth study was conducted. This study was designed to be more powerful than the previous study and, although there are statistical errors, as reported in the attached Statistical Appendix, the study accomplished this goal.

The follow-up seedling growth study did not show the same effects as the first study and, in particular, the adverse dose related trends in corn and soybean plant height and lettuce root length, observed in the first study, were not observed. There is an adverse effect reported on elongation percentages for corn at 1000 ppm. However, this effect is different than the effect observed in the first study and corresponding effects in the actual height data for corn, that are used to calculate the elongation percentage, were not observed. Therefore, the biological significance of this effect is questionable. Given the lack of repeatable effects, the high concentrations at which any effects are observed and the questionable biological significance of the effect on corn elongation percentage, the data indicate that, at the level of introduction, no significant effects from copoly VP/ST would be expected in plants.

The dungworm study was conducted in 1985 and there were significant procedural difficulties with the study. Although there were sublethal effects reported in the study associated with the flaccidity of the worms, no significant dose related mortalities were observed and the information presented in the study indicates that copoly VP/ST does not appear to be very acutely toxic to the species of worm tested. Based upon this information, copoly VP/ST is not expected to have any significant acute toxic effects to dungworms. As this species is considered to be representative of terrestrial invertebrates, these data indicate that copoly VP/ST would not have a significant impact on these organisms.

002637

Based upon the information provided in the EA, the Center concludes that copoly VP/ST and, consequently, RPAA is not expected to have a significant impact on the environment.

12/13/91
Date

Charles E. Goshen
Chief and Preparer
Environmental Sciences Staff
HFV-152

12/13/91
Date

Shawn A. Kilduff
Primary Action Officer
HFV-226

Attachments

June 13, 1991, Statistical Appendix
May 14, 1990, Environmental Assessment Vol. I - II