Environmental Assessment

Gentian Violet in Animal Feeds

I. Description of the Proposed Action

A. Proposed action and regulatory authority

The Food and Drug Administration's (FDA's) Center for Veterinary Medicine (Center) is proposing to declare gentian violet a food additive and a new animal drug when added to animal feed. This proposal would revoke FDA's interim policy that sanctions the use of gentian violet at up to 8 parts per million (ppm) as a mold inhibitor in poultry feed. There are currently no approved uses of gentian violet in animal feed and therefore the proposed action, if implemented, would prohibit the use of gentian violet in animal feed until a food additive regulation for such use is promulgated by the agency or a new animal drug application for such use is approved by the agency.

This action is being taken because the FDA has determined that gentian violet is not generally recognized as safe (GRAS) and is a food additive subject to section 409 of the Federal Food, Drug, and Cosmetic Act (the act), 21 U.S.C. 348, and is not generally recognized as safe and effective as an animal drug and is a new animal drug subject to section 512 of the act.

B. Underlying purpose and need for the proposed action

Gentian violet (methylrosaniline chloride) is a mixture of crystal violet (hexamethylpararosaniline chloride) and methyl violet (pentamethylrosaniline chloride). These compounds belong to a class of dyes related by molecular structure known as di- and triaminophenylmethanes. Gentian violet U.S.P. must be at least 96% crystal violet.

Since the 1960s, gentian violet has been marketed for use in inhibiting mold and fungus growth in animal feed. The FDA has never approved any product containing gentian violet for use in animal feed. In the past, the FDA has stated that gentian violet is not generally recognized as safe (GRAS) for use in animal feed and that it would require an approved food additive regulation or new animal drug application before the substance can be marketed for animal use. The FDA has taken regulatory action against gentian violet or the firms marketing gentian violet whenever the agency became aware that this substance was being marketed for use in food animals. The FDA's position that gentian violet is not GRAS for any use in food animals has been upheld in several court opinions or orders (see, for example, United States v. Naremco, Inc., 553 F. 2d 1138 (8th Cir. 1977)).

In the FEDERAL REGISTER of March 30, 1979 (44 FR 19035), the FDA denied a food additive petition that proposed the establishment of a regulation to permit the safe use of gentian violet. In denying the petition, the FDA

stated its concerns about the safety of gentian violet (i.e., its potential carcinogenicity, its similarity in chemical structure to known animal carcinogens, and its genetic toxicity in several in vitro and in vivo assays). Based upon these concerns, the FDA concluded that gentian violet was not shown to be safe for use as an ingredient of animal feed and that anyone wishing to market the substance would need to establish its safety before the substance could be legally sold.

However, in 1981, in a case involving a gentian violet poultry premix, a jury decided that gentian violet was indeed GRAS when used as a mold inhibitor at not more than 8 ppm in poultry feeds and, therefore, was not a food additive within the meaning of the act (see United States v. Article of Food ... Gentian Violet, No. C78-63G (N.D. Ga. April 15, 1981)).

Because of this finding, the FDA decided that it would not take further regulatory action against this use of gentian violet, even though the FDA continued to think that gentian violet was not GRAS for use in food animals. The FDA announced this decision in the FEDERAL REGISTER of July 27, 1982 (47 FR 21480) and stated that its decision in no way authorized any other use of gentian violet in food animals. In this announcement the FDA also stated that it had commissioned studies to test the safety of gentian violet and that it would re-evaluate its position on gentian violet when this new evidence became available.

The FDA has received reports from the National Center for Toxicological Research (NCTR) for several studies that the NCTR conducted to test the safety of gentian violet. The studies by the NCTR demonstrate that gentian violet is a carcinogen in mice and that significant residues of gentian violet occur in the edible tissues of chickens fed gentian violet. The FDA has reviewed these NCTR studies and concurs with the findings.

Therefore, the FDA has re-evaluated the prior position sanctioning the use of up to 8 ppm gentian violet in poultry feed as a mold inhibitor and concludes that: 1) gentian violet is not GRAS for this use, 2) this substance is a food additive when used in poultry feed as a mold inhibitor, and 3) all use of gentian violet in poultry feed must stop until safe conditions of its use can be identified and a food additive regulation promulgated or a new animal drug application approved.

II. Environmental Consequences of the Proposed Action

A. Use for which sanction would be revoked

Hagler (1983) and Tindall (1983 a&b) list several methods for preserving feed ingredients and they also briefly summarize the need for and uses of preservatives, especially mold inhibitors, in animal feeds. Propionic acid, a naturally occurring volatile organic acid, would appear to be the main chemical used as a mold inhibitor in animal feeds. Other naturally

occurring organic acids (e.g., benzoic acid, sorbic acid) and the salts of these acids (e.g., calcium propionate, sodium benzoate, potassium sorbate) are also used alone, or in various combinations, to preserve animal feeds. Gentian violet is listed by Tindall (1983a) for use only as a mold inhibitor in poultry feed.

The proposed rule, when finalized, would no longer allow the use of gentian violet in poultry feed, until such time as the chemical can be shown to be safe when used in this manner. Gentian violet is only one of several chemically active ingredients that are currently being used in products marketed as mold inhibitors for animal feeds (Hagler, 1983; Tindall, 1983 a&b). Several organic acids and their respective salts are already listed by the FDA as GRAS items that can be used as a general purpose food additive or as a chemical preservative (21 CFR 582 B and D). Therefore, it can be expected that the other chemicals already used for this general purpose, such as the organic acids and their salts (especially propionic acid and its salts, sodium propionate and calcium propionate), will substantially replace the existing use of gentian violet in poultry feed.

B. Magnitude of use for which sanction would be revoked

The introductions of chemicals into the environment will therefore be affected by this proposed rule. The manufacture, use, and disposal of products and poultry feed containing gentian violet currently result in residues of this chemical being introduced into the environment. The magnitude of the use of gentian violet in poultry feeds will therefore be estimated so that the potential increase in environmental introductions of the probable chemical replacement for this use of gentian violet in poultry feeds, propionic acid, can then be approximated.

After conversations with poultry farmers, manufacturers, university researchers and USDA officials, the FDA assessed the economic effects attributable to the proposed regulation (FDA, 1986). The following assumptions are pertinent to estimating the magnitude of use for gentian violet or its replacements and these assumptions were either taken directly from, or derived from, data present in the December 5, 1986, FDA economic threshold assessment (FDA, 1986).

1. From about 30% to 60% of all poultry feeds are now routinely mixed together with mold inhibitors.

2. The share of this mold inhibitor market for gentian violet ranges from about 10% to 40% of that total (i.e., from 3% to 24% of all poultry feeds are routinely treated with gentian violet).

3. About 35 million tons of poultry feeds are manufactured each year in the United States. [This estimate was based upon U.S. poultry production figures (USDA, 1986) and poultry feed consumption figures. This estimate of 35 million tons agrees well with that of 36.3 million tons by Mr. Dennis Jackson of Cambridge Products Ltd., a manufacturer of mold inhibitors].

4. Mold inhibitor products containing gentian violet contain 1.6% of this chemical as the active ingredient.

5. Products containing propionic acid will be the most common replacement for gentian violet and these products commonly contain about 50% propionic acid as the active ingredient.

6. Mold inhibitor products will be used at a uniform rate of 1 lb product/ton of poultry feed. [Propionic acid use rates of up to 2-3 lbs/ton may be needed when feeds have a high moisture content].

The potential market for the use of gentian violet in poultry feed has been estimated to be about 35 million tons of poultry feed. This, along with other assumptions above, allow for an estimate of the range of the current use of gentian violet (active ingredient) in poultry feeds at from 16,800 lbs/yr to 134,400 lbs/yr.

Calculation:

Assumption 2 (3% to 24% of poultry feeds treated with gentian violet)
35 million tons/yr X 3% = 1.05 million tons/yr treated with gentian violet
35 million tons/yr X 24% = 8.4 million tons/yr treated with gentian violet

Assumption 6 (1 lb product with gentian violet used per ton of feed)
1.05 million tons/yr X 1 lb product/ton = 1.05 million lbs product/yr
8.4 million tons/yr X 1 lb product/ton = 8.4 million lbs product/yr

Assumption 4 (products contain 1.6% gentian violet)
1.05 million lbs product/yr X 1.6% = 16,800 lbs gentian violet/yr
8.4 million lbs product/yr X 1.6% = 134,400 lbs gentian violet/yr

C. Magnitude of the replacement use

Use of the same procedure (with the replacement of Assumption 4 with Assumption 5) allows for an estimate of the range of possible additional use of propionic acid that could result from the substitution of propionic acid for gentian violet in poultry feeds. The potential increased use of propionic acid therefore ranges from 0.52 million to 4.2 million lbs/yr.

Calculation:

As above, except Assumption 5 replaces Assumption 4

Assumption 5 (products with propionic acid contain 50% active ingredient)
1.05 million lbs product/yr X 50% = 0.52 million lbs propionic acid/yr
8.4 million lbs product/yr X 50% = 4.2 million lbs propionic acid/yr

D. Significance of potential shift in chemical introductions

The magnitude of these presumed changes in the annual use (and potential environmental introductions) of gentian violet and propionic acid in the United States also needs to be placed in perspective with the total annual production of these two chemicals in the United States.

The following table shows the total U.S. production of Basic Violet 3 (crystal violet, the primary ingredient in gentian violet) and propionic acid over the last three years for which data are available (USITC, 1984; 1985; 1986):

	Basic Violet 3 (million lbs)	Propionic Acid (million lbs)
1983	1.097	101.4
1984	0.997	95.9
1985	1.117	99.7
Average	1.07	99.0

Proportionally, the estimated range of gentian violet use in poultry feeds represents from 1.6% to 12.6% of the average total annual production of Basic Violet 3. This would represent the estimated production decrease of this dye, should the proposed action be implemented and the use of gentian violet in poultry feeds no longer be permitted.

16,800 lbs/yr / 1.07 million lbs/yr = 0.016 = 1.6%
134,400 lbs/yr / 1.07 million lbs/yr = 0.126 = 12.6%

Similarly, a comparison of the estimated range of the potential increases in the use of propionic acid in poultry feeds with the three year average in production of this chemical yields an estimated increase in production from about 0.5% to 4.2% of the annual production of propionic acid. This would represent the estimated increase in the production of this organic acid, should the proposed action be implemented.

0.52 million lbs/yr / 99 million lbs/yr = 0.005 = 0.5%
4.2 million lbs/yr / 99 million lbs/yr = 0.042 = 4.2%

Therefore, it can be concluded that, based upon total Basic Violet 3 production in the United States, a revocation of the use of gentian violet in poultry feeds is unlikely to significantly affect the total market for this dye. Similarly, it can be concluded that, based upon total propionic acid production in the United States, an increase in the use of this chemical in poultry feeds is unlikely to significantly affect the total market for this organic acid.

E. Environmental impact of the proposed action

The Center has considered environmental information for gentian violet (Appendix A), the probable replacement chemical, propionic acid (Appendix B), and one other less likely replacement chemical, benzoic acid (Appendix C) and concludes that the proposed rule will not have a significant impact on the quality of the human environment.

The potential environmental impact associated with the reduced use of gentian violet or increased uses of organic acids (primarily propionic acid) is not expected to be significant. This conclusion is based upon the following analysis and the data present in the attached appendices.

The current use of gentian violet in poultry feed does not appear to be causing adverse environmental effects at the sites of use (see Appendix A). Most of the gentian violet in poultry diets is ultimately excreted in the wastes from these animals. The concentration of gentian violet residues expected in poultry wastes is estimated to be about 9.5 ppm (mg residue/kg waste). About 60% of the gentian violet residues in the poultry excreta are expected to be parent compound, with about another 30% expected to be demethylated metabolites and perhaps a small proportion of leukogentian violet. It is common for the excreta from poultry to be kept for extended periods of time (a year or longer) in the confined poultry facility until it can be used as a fertilizer added to agricultural soils.

The approximate maximum level of total gentian violet residues expected in soils amended with poultry wastes from treated animals is about 0.07 ppm, a level that would probably not be expected to cause acute effects in organisms living in the terrestrial environment. Due to the moderate to strong tendency for gentian violet to sorb to particulate and organic matter, the residues of gentian violet and its metabolites are expected to stay primarily in the soils amended with the poultry waste. The ability of light and of some chemicals and microbes to degrade these compounds should allow for the degradation and eventual loss of gentian violet residues from the amended soils.

The transfer of gentian violet residues from the above soils amended with poultry wastes into closely located aquatic environments would appear to be rather unlikely. Regardless of that likelihood, an extreme example of the potential transfer of gentian violet residues into the aquatic environment via runoff from a terrestrial location during a heavy rain storm indicates that the maximum levels that could be expected in the runoff itself are about 0.3 ppm (mg/L). Runoff from rainfall is usually further diluted somewhat by the volume of water receiving this input (e.g, a stream, pond, lake, ocean). The maximum possible levels of gentian violet residues that could enter the aquatic environment via runoff are expected to be below the levels that have been demonstrated to result in effects in organisms that live in the aquatic environment.

The environmental impacts associated with the manufacture of gentian violet dye, mold inhibitor product, and treated poultry feeds are unclear. The documents that examine dye manufacture have identified several areas of potential concern without resolving whether or not environmental impacts are expected or have occurred. It is likely that the dye industry has made some progress in the reduction and treatment of the wastes that are emitted by their facilities. The proposed rule would, however, eliminate occupational exposures to and other environmental releases of gentian violet at sites where mold inhibitor product and treated poultry feeds are prepared.

The above analysis on the potential environmental impacts of the use of gentian violet in poultry feeds indicates that the current environmental impacts would appear to be minor.

There are adequate products that are currently widely used for the same use as gentian violet. The active ingredients in the products evaluated in this document pose little potential for causing adverse environmental impacts. Both propionic acid and benzoic acid are naturally-occurring compounds that are found widely in the environment. Both of these organic acids appear to be a normal component of the metabolism and excretion of a wide number of organisms present in the environment (see Appendices B and C).

The uses of propionic acid and benzoic acid (and their salts) as preservatives in food and the use of propionic acid in animal feeds is already common. The use of these organic acids in poultry feed would not be expected to result in a significant increase in environmental impacts.

Propionic acid has been found to be a natural component of animal wastes and is a metabolic end product of many anaerobic bacteria. It is also a common component of sewage and has been routinely found in the effluent of wastewater treatment plants. This compound is readily biodegradable and is utilized as an energy source by all manners of aerobic bacteria and higher organisms. Benzoic acid has also been found to be a natural excretion product of a wide variety of biota. It has also been found in sewage and in the effluent of wastewater treatment plants. Both compounds are as degradable as are sugars and can be utilized as nutrients by diverse organisms. Therefore, both of these compounds would not be expected to present any additional significant environmental impacts when used as a preservative in poultry feeds.

The environmental impact of the proposed rule on the utilization of resources and energy is not expected to be significant. As previously noted, the production of gentian violet for use in poultry feeds represents a small percentage of the total production of this dye. Therefore, the reduction in the production of gentian violet that would result from this action should not result in any significant changes in the use of resources and energy.

Similarly, the total production of both propionic acid and benzoic acid should not be significantly affected by the relatively small increases in production that would result from this action. Any increase in resource and energy utilization resulting from an increased production of these organic acids may be expected to be offset by the decrease in production of gentian violet. Overall, changes in resource and energy utilization will not be expected.

III. Mitigation Measures

It is possible that a cessation of the use of gentian violet in poultry feeds may result in the need for the disposal or appropriate re-use of gentian violet product and feeds containing gentian violet. In such a case, any product or feed, whether held privately, recalled, or enjoined or seized by the FDA, must be re-used, recycled or disposed of in accordance with Federal, State or Local requirements. The most applicable requirements would appear to be those governing solid and hazardous wastes. Solid and hazardous waste programs are administered largely by the States and, based on past experience, requirements are expected to vary from State to State. Where necessary, the Center Environmental Staff can be contacted (301/443-1880) for referrals to the appropriate State authority.

Surprisingly, given the long history of use of gentian violet, the scientific literature does not appear to contain much guidance on the proper disposal of this dye. The physical-chemical and environmental fate properties of the dye, as described in Appendix A, also do not suggest a clear-cut method for disposal of gentian violet. Aldrich (1986) recommends that gentian violet (entry # 86,099-9) be dissolved or mixed with a combustible solvent and burned in a chemical incinerator equipped with an afterburner or scrubber. This method would appear to be most applicable to mold inhibitor products containing gentian violet.

For feeds treated with gentian violet amendment into agricultural soils as a soil conditioner would appear to be one possible disposal technique to be considered. The concentration of the dye present is approximately equal to the concentration present in manure from treated poultry, which has been amended into agricultural soils for many years. Some microorganisms have been reported to slowly degrade gentian violet. Gentian violet has been reported to be sensitive to light. The dye is also reported to sorb to activated carbon and to particulate material. Appendix A contains reviews of the pertinent environmental fate information. Whether this technique or some other procedure would be used would be determined by individual State authorities.

IV. Regulatory Alternatives and the Expected Environmental Consequences.

The proposed action declares that gentian violet used in poultry feeds for mold inhibition is not generally recognized as safe and that an approved food additive petition or new animal drug application is required for the marketing of the product. This type of action is not addressed specifically in the FDA's procedures (21 CFR 25) implementing the National Environmental Policy Act and was therefore addressed on a case-by-case basis under 21 CFR Part 25.22(a)(19). An environmental assessment was prepared because this action would remove gentian violet from the marketplace, at least until a FAP or NADA was approved for such products. From an environmental viewpoint, this would mean that there would be potential for increased use of alternate products and, perhaps, the need to dispose of some gentian violet products and treated feeds through environmentally acceptable methods. Due to the fairly widespread use of gentian violet in poultry feed, the Center decided to examine the environmental properties of gentian violet and its replacement products and to determine, if possible, appropriate measures for the disposition of products and feeds containing gentian violet, as discussed above in III.

The proposed action will either be finalized or no action will occur. There is no need to examine other regulatory alternatives because no adverse environmental impacts were found for the proposed action. See 21 CFR 25.41(a).

No Action: If, during the comment period for the proposed rule, convincing evidence is provided to affirm that gentian violet is not a food additive or new animal drug for the affected use of gentian violet, then it is conceivable that No Action will occur. In such a case, no change in present environmental introductions during the manufacturing and use of the product will be expected. Based on the limited data available, the current use of gentian violet in poultry feeds would not appear to be the cause of significant environmental impacts.

V. List of Preparers of this Document

Maurice Zeeman, Ph.D. was the primary preparer of this EA and Appendix A. Dr. Zeeman has been an environmental toxicologist with the Center's Environmental Staff since 1980. He is responsible for the analysis of the potential environmental impacts of actions proposed by the Center, for providing guidance to applicants on the types of environmental data needed to determine whether a proposed action requires the preparation of an environmental impact statement, and for the evaluation of environmental documents prepared by other agencies or by individuals. Dr. Zeeman has earned an M.A. degree from U.C.L.A. in Biology (Ecology) and a Ph.D. degree from Utah State University in Zoology (Toxicology). He is a member of several scientific societies; is the author of several scientific publications in the discipline of environmental toxicology; is appointed to the Publications Committee for the Society of Environmental Toxicology and Chemistry; and in 1982 became an Adjunct Professor at the NIH Graduate School, where he teaches Environmental Toxicology.

John C. Matheson III prepared portions of this EA and prepared Appendix B. Mr. Matheson has served as an environmental scientist at FDA for eleven years, the last six and one-half as FDA's NEPA focal point and as Chief of the Center's Environmental Staff. Mr. Matheson earned his MSPH in Environmental Sciences and Engineering (1975) and a B.S. in Biology (1973) at the University of North Carolina-Chapel Hill.

Charles E. Eirkson III prepared Appendix C. Mr. Eirkson has been an environmental biologist with the Center's Environmental Staff for over two years. He is responsible for the analysis of the potential environmental impacts of actions proposed by the Center, for providing guidance to applicants on the types of environmental data needed to determine whether a proposed action requires the preparation of an environmental impact statement, and for the evaluation of environmental documents prepared by other agencies or by individuals. Mr. Eirkson earned his B.S. in Biology with specialization in biogeography and environmental science at the University of Pittsburgh, Pittsburgh, Pennsylvania (1975) and is currently preparing a thesis for the fulfillment of the requirements for his Masters degree in Environmental Science at Hood College, Frederick, Maryland.

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VI. References

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VII. Appendices

Attached find Appendix A (Environmental Assessment Data for Gentian Violet), Appendix B (Environmental Assessment Data for Propionic Acid), and Appendix C (Environmental Assessment Data for Benzoic Acid).

Appendix A: Environmental Assessment Data for Gentian Violet

1. Chemical identity and properties.

a. Common names (The Merck Index, 1983):

Crystal violet
C.I. Basic Violet 3
C.I. 42555
methylrosaniline chloride
hexamethylpararosaniline chloride

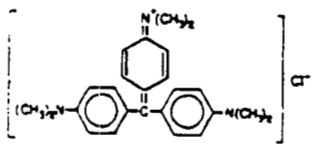
U.S.P. grade gentian violet contains not less than 96% gentian violet (crystal violet, Basic Violet 3).

b. Chemical name (USAN & USP, 1984):

Methanaminium, N-[4-[bis[4-(dimethylamino)phenyl]methylene-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride

c. CAS registry number: 548-62-9 (USAN & USP, 1984)

d. Structural diagram (USAN & USP, 1984):



e. Empirical formula: $C_{25}H_{30}ClN_3$ (USAN & USP, 1984)

f. Molecular weight: 407.99 (USAN & USP, 1984)

g. Physical description: Dark green or greenish powder. Crystalline pieces glisten with a metallic luster (The Merck Index, 1983).

2. Introduction into the environment through manufacture.

During 1985, 1.2 million pounds of Basic Violet 3 was produced in the U.S. by four manufacturers. (USITC, 1986). The primary use of gentian violet is probably as a dye in textiles and paper, however, it is also used as a biological stain, a pH indicator, a pesticide, and as a mycostatic agent in medicine and agriculture (EPA, 1981; McDonald and Cerniglia, 1984; Rushing and Bowman, 1980; The Merck Index, 1983).

Brown et al. (1981) report that the estimated losses during the production of dyes and pigments is often in the range of 1-2%. They also state that "these losses are largely into the aqueous effluents arising from the production or use industry." Brown (1987) estimates that about another 9% of dyestuffs is discharged as a result of the actual dyeing operations.

Crystal violet (Basic Violet 3) represents a very small proportion of the total synthetic organic dyes produced in or imported into and used in the United States. In 1980, 245 million lbs (111 million kg) of synthetic organic dyes were produced in the U.S. and an additional 29 million lbs (13 million kg) were imported (Kulkarni et al., 1985). In contrast, the production of Basic Violet 3 in the U.S. over the period 1983 to 1985 averaged only about 1.1 million lbs (0.5 million kg) per yr (USITC, 1984; 1985; 1986), and in 1979, only 32,000 lbs (14,500 kg) of gentian violet were imported into the U.S. (Swett et al., 1984).

Gentian violet is a triarylmethane dye and these dyes are among the oldest synthetic dyes (Gwinn and Bomberger, 1984). Crystal violet was discovered by Kern and Caro in 1883 (Colour Index, 1971). This dye is manufactured by reacting N,N-dimethylaniline with phosgene in the presence of zinc chloride to give Michler's Ketone [4,4'-bis(dimethylamino)benzophenone]. This is further condensed with dimethylaniline to yield crystal violet (Gwinn and Bomberger, 1984).

Bomberger et al. (1984) summarized the publications examining the wastes from the manufacture of dyes and pigments. One of these publications (Gwinn and Bomberger, 1984), specifically reviewed the wastes expected from the manufacture of 48 diphenylmethane and triarylmethane dyes and pigments. The wastes from the manufacture of crystal violet and similar triarylmethane dyes were discussed in a general fashion. Gwinn and Bomberger (1984) identified the following manufacturing waste streams that could contain hazardous materials (such as phosgene, heavy metals, Michler's Ketone, etc.) as a result of the various processes involved in making triarylmethane dyes:

a. Shipping containers, discarded
b. Solid residues from synthesis, distillation and filtration
c. Gaseous emissions
d. Process wastewaters
e. Wastewater treatment solids

Gwinn and Bomberger (1984) identify the following materials as being released into the environment as a result of the waste streams that are involved in the manufacture of crystal violet:

a. Basic Violet 3
b. Phosgene
c. N,N-dimethylaniline
d. 4,4'-bis(dimethylamino)benzhydrol
e. 4,4'-bis(dimethylamino)benzophenone [Michler's Ketone, a substance that is carcinogenic in experimental animals (NTP, 1986)].

Gwinn and Bomberger (1984) state that significant sources of hazardous wastes may be found in the process sludges, solid distillation residues and gaseous emissions, however, the authors think that the major potential source of emissions would be from the process wastewaters,

primarily because it contains the mother liquor (with unreacted intermediates, by-products, dye, etc.). These authors conclude by stating that: 1) different dye producers have different wastewater treatment systems, 2) several triarylmethane dyes inhibit aerobic wastewater bacteria, 3) there is a good chance that many aerobic treatment units will not degrade the basic dyes and their intermediates, 4) unreacted dye may remain on wastewater solids (sludge), and 5) these solids may be further treated in anaerobic digesters or deposited in the anaerobic environment of a landfill.

Rushing and Bowman (1980) attempted to recover and analyze gentian violet added to industrial wastewaters. In this experiment the authors percolated 15 liters of industrial wastewater containing 1 ppm (part per million) gentian violet through an activated carbon column. This was done to simulate a a large-scale process used at the National Center for Toxicological Research (NCTR) for the removal of hazardous residues from industrial wastewater prior to its discharge into the environment. The authors found that the activated carbon column (but not a column packed with XAD-2 resin) efficiently removed the gentian violet, with no residue (<20 parts per billion (ppb)) emerging from the column.

Clarke and Anliker (1984) generally discuss the safety aspects of producing dyes and pigments. They conclude that workers in the dye and pigment industries should: 1) be aware of the hazardous properties of these chemicals (e.g., via safety data sheets), 2) be advised of precautions for their safe handling, 3) minimize exposures to these chemicals, and 4) avail themselves of medical surveillance.

It may be difficult for workers exposed to gentian violet to know how hazardous this chemical may or may not be in the workplace. In 1984, Swett et al. report looking for TLV's (Threshold Limit Values) used to limit worker exposure to colorants in the workplace air in regulations for OSHA (Occupational Safety and Health Administration) and in standards recommended by NIOSH (National Institute for Occupational Safety and Health) and the ACGIH (American Conference of Governmental Industrial Hygienists), but no standards or limits could be found. Similarly, Kulkarni et al. (1985) report that little information on worker exposure to dyestuffs exists, except for the benzidene-based dyes.

In the draft Chemical Hazard Information Profile (CHIP) prepared for gentian violet by EPA in 1981, the EPA (1981) also found that "gentian violet is not currently the subject of existing or recommended standards or regulations." However, this report also acknowledges that some workers producing rosaniline dyes, such as gentian violet, have been instructed to wear respirators. This was because nosebleeds in dye manufacturing plants occurred when workers did not wear respirators. Epidemic nosebleeds were also reported in four other industries where workers were exposed to gentian violet (EPA, 1981). EPA (1981) also reports that "gentian violet is moderately acutely toxic, a severe eye irritant, a mucous membrane irritant, and a potent inducer of chromosomal aberrations..."

Lawrence, and Smith (1982) report that gentian violet has resulted in very few reported cases of contact sensitivity, despite its wide use for many years as a medicament. Bajaj and Gupta (1986) report that gentian violet is a mild skin sensitizer. EPA (1981) states that skin contact with gentian violet can cause eczema, acneform eruption and papillary growth.

3. Introduction into the environment through use in animal feeds.

Gentian violet is being used at a level of up to 8 ppm (parts per million) as a mold inhibitor in poultry feed (Hagler, 1983; Tindall 1983 a&b). Gentian violet that is ingested along with the feed should only be absorbed to a small extent (McDonald and Cerniglia, 1984) and is excreted fairly rapidly by poultry, primarily as parent gentian violet, along with some metabolites (McDonald and Cerniglia, 1984; McDonald et al., 1984; McDonald, 1985). These gentian violet residues end up in poultry excreta, which is often amended to soil as a fertilizer.

Most poultry in the U.S. are raised in confinement in large operations and the excreta from these birds are usually collected for extensive periods before they are taken from the confined area and applied to or amended with soils (White and Forster, 1978). Therefore, as a result of this use of gentian violet in poultry feeds, the primary introduction of gentian violet into the environment should be into soils amended with poultry wastes. Estimates are given below of the concentration of gentian violet (and metabolites) expected in poultry excreta and in soils amended with these wastes.

The following estimate is based upon data from EPA (1974) on raising broiler chickens and the wastes that result from this type of operation. In 6-8 weeks, chicks weighing about 5 g become marketable broilers weighing an average of 1.8 kg. Over that time period, the average bird is fed 0.064 kg feed/day and excretes 0.054 kg of raw waste/day. That weight of feed with 8 ppm of gentian violet results in a daily intake of gentian violet of 0.512 mg/bird. Assuming that essentially all of the gentian violet is excreted intact, the gentian violet concentration in the wet poultry wastes should be no higher than about 9.5 ppm (0.512 mg gentian violet/0.054 kg waste = 9.5 mg/kg = 9.5 ppm).

The rate of poultry excreta amendment to soils will vary depending upon several circumstances (e.g., soil type, rainfall, etc.). For the purpose of this assessment, a maximum practical application rate of 7.5 tons/acre (16.8 metric tons/hectare) will be used (Fuller and Warrick, 1985).

The top six inches (15.2 cm) of soil is the part that is normally amended with animal wastes. That depth of soil in a one acre plot weighs about two million lbs (909,000 kg). Therefore, that depth of soil in a one hectare (ha) plot (ha = 2.47 acres) would weigh about 2.25 million kg. [One metric ton = 1,000 kg (2,200 lbs)].

Chicken wastes incorporated into soil at 16.8 metric tons/ha would result in a total of 0.16 kg of gentian violet being added to the top 15.2 cm of each hectare of soil (9.5 mg gentian violet/kg waste X 16,800 kg waste/ha = 159,600 mg gentian violet/ha = 0.16 kg gentian violet/ha). This is equivalent to 0.14 lb of gentian violet/acre of soil (0.16 kg/ha X 2.2 lb/kg = 0.35 lb/ha X 0.405 ha/acre = 0.14 lb/acre).

The relative concentration of gentian violet in top soil amended with such chicken wastes would be about 0.07 ppm (159,600 mg gentian violet/2.25 million kg soil = 0.071 mg/kg = 0.07 ppm).

The gentian violet that is introduced into soils could stay primarily in the terrestrial environment. This is because gentian violet appears to have chemical characteristics that would tend to keep it in that environmental compartment (e.g., moderate to large octanol/water partition coefficient (Kow), strong binding to particulate and organic matter and to soil anions).

Nevertheless, gentian violet and its metabolites might enter the aquatic environment. To estimate the maximum possible concentration of gentian violet that might occur in runoff from soils amended with poultry wastes, the following scenario is postulated. If it rains heavily before the incorporation into soil of poultry waste containing 9.5 ppm of gentian violet, the maximum concentration which could be found in two inches of runoff (two inches of water per hectare weighs 507,585 kg) would be approximately 0.3 ppm (159,600 mg gentian violet/ha divided by 507,585 kg water/ha = 0.31 mg/kg = 0.31 ppm).

It should be recognized that these estimated concentrations of gentian violet in soil and runoff are high relative to the amounts which may actually be present in the environment. This is because (1) a high initial concentration in poultry wastes was utilized, (2) the initial concentration in wastes did not include the mass of bedding which is normally utilized by poultry producers, (3) poultry wastes are usually stored for a period before spreading onto soils resulting in degradation of waste residues, and (4) it is unlikely that all of the gentian violet residue would be present in either soil or runoff, instead, the residue would be distributed between these two compartments. In addition, any gentian violet in the runoff would be further diluted by the body of water receiving the runoff.

4. Fate of gentian violet in the environment.

   a. Water solubility: Gentian violet is soluble in cold and hot water (Colour Index, 1971). U.S.P. grade gentian violet is reported to be soluble in an aqueous medium at 1:30 (water solubility of about 33,000 ppm).

b. UV-visible absorption maxima:

588 nm (Rushing and Bowman, 1980)
590 nm (Green et al., 1979)
592 nm (Idaka et al., 1985)
600 nm (Kwasniewska, 1985)

c. Octanol/water partition coefficient (Kow): Kow >100 (Yatome et al., 1981 a&b)

d. Dissociation constant: Crystal violet is almost completely ionized over the pH range of 5 to 9 (Moats and Maddox, 1978) and they report that the calculated pK = 9.36 (fide Goldacre and Phillips, 1949).

e. Sorption/desorption: Pagga and Brown (1986) tested 87 dyes for biodegradation and found that "with many dyestuffs a substantial colour removal was observed which may be attributed to the elimination of the dyes by adsorption." Similarly, Rushing and Bowman (1980) attempted recoveries of gentian violet added to industrial wastewater and found erratic results due to "the adsorption of the gentian violet onto particulate matter present in the wastewater." Brown (1987) says that many dyestuffs "may be expected to be removed from an effluent by sorption onto sludges during sewage treatment..."

Michaels and Lewis (1985) report that they have determined the sorption of Basic Violet 3 to freshwater microorganisms (lab and field collected samples of bacteria and algae). The partition coefficients that they report range from 1,200 to 14,000 for live organisms and 520 to 1,500 for heat killed organisms. They report that the dyes that they tested "sorb to bacterial cells at a much slower rate than that which has been reported for pesticides" with the sorption of Basic Violet 3 to viable cells taking about 24 hrs to reach equilibrium. They also note that the sorption values (Kd) for the violet dyes indicate a greater sorption to the gram-positive as compared to the gram-negative organisms. Moats and Maddox (1978) tested the effects of pH on crystal violet antimicrobial activity. They found that crystal violet would be expected to remain almost completely ionized over the pH range of 5 to 9. Therefore, sorption of the positively charged form of gentian violet to anions in soils might be predicted in this pH range.

McDonald and Cerniglia (1984) found that the intestinal microflora of several species (humans, rats, and chickens) metabolized gentian violet to leukogentian violet. Attempts at extraction of this metabolite from microfloral cultures indicated that the major portion of the metabolite was bound to the cells and not found in the supernatant.

Kwasneiwska (1985) reports the confirmed presence of crystal violet in the sediment and water of the Buffalo River, near Buffalo, N.Y. This report was based upon the study by Nelson and Hites (1980). Diachenko (1979) found several aromatic amine compounds in Buffalo River fish which may have resulted from the manufacture of triarylmethane dyes and he was concerned about the potential for biomagnification by fish of these compounds. Shortly thereafter, Nelson and Hites (1980) took sediment samples from the Buffalo River and soil samples near its bank to determine if a nearby dye manufacturing plant might be the source of this contamination.

Nelson and Hites (1980) found that they could identify 11 aromatic amines in both the sediment and soil samples that were related to the commercial production of crystal violet and malachite green. The leuko forms of both gentian violet and malachite green were two of these identified compounds. The high concentrations of various aromatic amines in the soil samples caused Nelson and Hites to suggest that "this particular site may have been used as a chemical dump by the dyestuff manufacturer." These authors say that structural similarities and concentration ratios of these compounds suggest that these chemicals may have entered the water and sediment of the river via runoff and leaching from the chemical dump. Nelson and Hites conclude that some of these compounds could be mutagenic or carcinogenic and therefore may possibly be responsible for the tumors observed in fish taken from the river (Black et al., 1980).

f. Degradation: One common means used to detect the "degradation" of gentian violet is via the decolorization of solutions or media containing gentian violet. In some cases this decolorization probably represents a significant degradation of the gentian violet molecule (Green et al., 1979). However, in several other cases presented below, the degradation reported may simply represent a transformation of the colored gentian violet into its colorless leuko form. Note that upon the establishment of appropriate chemical conditions, the leuko form of gentian violet (and of the chemically closely related triarylmethane dye, malachite green) can be readily converted back into the parent compound (Poe and Wilson, 1983; Reszka et al., 1986; Seltzer, 1986).

(1) Photodegradation: Rushing and Bowman (1980) mixed various levels of gentian violet into animal feeds and stored them either in the open exposed to light for 16 days, or in amber bottles in the dark for up to 17 weeks. These researchers found that the levels of gentian violet in the feed decreased under both conditions. They concluded that the gentian violet residues in animal feed decline markedly due, in part, to photodegradation and/or oxidation. They then exposed gentian violet (in methanol) to incandescent light for 19 days and no gentian violet residue was detected.

Docampo et al. (1983) found that gentian violet was reduced to the triarylmethyl free radical upon exposure to incandescent light and anaerobic conditions. This was found to be the same free radical as was formed by enzymatic reduction in parasitic protozoans (trypanosomes). This free radical was not found in the presence of air.

Reszka et al. (1986) also found that crystal violet was reduced to the triarylmethyl free radical by visible light and anaerobic conditions. They also found that, under aerobic conditions, there appears to be a redox cycling of the crystal violet free radical metabolite and the regeneration of the parent dye.

(2) Thermal degradation: Water bath temperatures in excess of 55°C will degrade gentian violet (Rushing and Bowman, 1980).

(3) Chemical degradation: Littlefield et al., (1985) claim that gentian violet is readily reduced to the colorless leuko form by a variety of inorganic reducing agents. In contrast, Kwasniewska, (1985) said that crystal violet is extremely stable and not affected by dilute acid, base, oxidizing, or reducing agents.

(4) Biodegradation: Clarke and Anliker (1984) acknowledge that, in general, "dyes do not readily undergo aerobic biodegradation and certainly not to any significant extent during passage through a biological effluent treatment plant." Similarly, Pagga and Brown (1986) tested 87 dyes in short-term biodegradation tests and confirmed that "dyestuffs are most unlikely to show any significant biodegradation in such tests."

The results of the studies specifically examining the biodegradation of gentian violet are quite diverse. Michaels and Lewis (1986) found that gentian violet was slowly degraded over a 15-day period by field isolates of aerobic microbes. They conclude that the toxicity of this persistent dye may decrease the overall mineralization of organic compounds, unless it is rapidly degraded by anaerobic populations.

In contrast, Kwasniewska (1985) found that crystal violet was rapidly degraded by two species of oxidative red yeasts (half life = t 1/2 = 2.5 to 3 days), however, the investigator also found that crystal violet was not degraded at all in 30 days by a fermentative yeast.

Crystal violet was found to be degraded by only three of the seven different strains of bacteria tested by Green et al. (1979). This decolorization of gentian violet did not appear to be due to a reduction (to the leuko form) or to result from enzymatic activity.

Basic Violet 3 was degraded by a "dye assimilating bacteria" with a t 1/2 of from 53 to 61 hours (Yatome et al., 1981 a&b). However, the same investigators found that Basic Violet 3 was not degraded sufficiently in four days for any t 1/2's to be calculated for the four strains of bacteria that they isolated from activated sludge (Yatome et al., 1984).

Gentian violet was metabolized primarily into leukogentian violet by human, rat, and chicken intestinal microflora and by nine genera of strict and facultative anaerobic bacteria (McDonald and Cerniglia, 1984). Leukogentian violet was not formed under aerobic or anaerobic conditions for two other species of facultative anaerobes.

The authors state that the metabolism of gentian violet by soil anaerobes and light could contribute to the leukogentian violet that was detected as an environmental contaminant in soils and sediments near a dye producing facility on the Buffalo River (see McDonald and Cerniglia, 1984, fide Nelson and Hites, 1980).

Gentian violet's major in vitro metabolites from the liver microsomes of chickens and several strains of rodents were stepwise demethylated analogs of gentian violet (McDonald et al., 1984). The demethylated analogs of gentian violet were the major metabolites detected in the excreta of chickens dosed orally with gentian violet (McDonald, 1985).

Gentian violet is reduced to a triphenylmethyl free radical metabolite by rat liver microsomes (Harrelson and Mason, 1982). Rat liver microsomes may also increase or decrease crystal violet activity against various bacterial strains used to detect mutagenicity (Thomas and MacPhee, 1984).

Leukogentian violet accounted for at least 11% of the residues found in the feces of a rat dosed orally with gentian violet (McDonald and Cerniglia, 1984).

5. Effects of gentian violet in the environment.

a. Microorganisms: Gentian violet has long been known to inhibit the growth of certain bacteria, fungi, and parasites (Docampo et al., 1983). These authors also report that it has also been used as an antiseptic for newborns and to control fungal and intestinal parasites in humans.

Moats and Maddox (1978) say that the triphenylmethane dyes, such as gentian violet, are "remarkably effective in inhibiting the growth of some types of bacteria" with the gram-positive bacteria, in general, being more sensitive than are the gram-negative bacteria. They report that gentian violet was one of the dyes they selected because it was "among the most inhibitory to microorganisms of a large number surveyed by various workers." These authors report that pH could have an effect on the dye concentrations that would permit growth of both dye resistant and dye sensitive organisms. At pH 7, the gentian violet concentrations permitting growth ranged from 0.1 to 1.0 ppm for the dye sensitive organisms and 5 to 10 ppm for the dye resistant organisms.

Michaels and Lewis (1985) also report that Basic Violet 3 has been used for a long time as an inhibitory agent in microbiological media. The bacteriostatic activity of triphenylmethane dyes, of which gentian violet appears to be one of the more active, is via interference with cell metabolism (Green et al., 1979).

McDonald and Cerniglia (1984) incubated the intestinal microflora of humans, rats, and chickens. They noted that gentian violet at a concentration of 2% ppm in the incubation medium did not inhibit bacterial growth.

Idaka et al. (1985) tested the effect of crystal violet upon the microorganisms present in the activated sludges of a wastewater treatment plant. They found that a crystal violet concentration of about 80 mg/L (ppm) affected the growth rate and tended to deactivate the sludge microbes. This effect on growth was reduced by repeated cultivation of the microbes in the presence of this dye.

Similarly, Brown et al. (1981) tested for the inhibitory effect of 202 dyestuffs on aerobic waste-water bacteria. They estimated the IC50 (concentration causing 50% inhibition of respiration) for Basic Violet 3 to be 10-100 mg/L (at three hours, 20% inhibition for 10 ppm and 80% inhibition for 100 ppm). Only 18 of the 202 dyes tested showed an IC50 of less than 100 ppm.

Methyl violet (Basic Violet 1) and malachite green (Basic Green 4) are two other triphenylmethane dyes that are both structurally very similar to gentian violet (Colour Index, 1971; Michaels and Lewis, 1985). These two dyes were tested for the American Dye Manufacturers Institute for effects upon aerobic and anaerobic wastewater treatment systems (Etzel and Grady, 1973; Hunter, 1973).

Hunter (1973) reports that both of these dyes were determined to be some of the most inhibitory towards aerobic microbial systems, in both wastewater treatment (activated sludge) and natural stream purification processes (biological oxidation of stream organic matter). These two dyes were tested at concentrations of up to 25 mg/L. Etzel and Grady (1973) found that Basic Violet 1, but not Basic Green 4, had a very definite initial inhibitory effect upon anaerobic digesters receiving primary sewage sludge. These authors report that this inhibition was seen "at a concentration of only 40 mg/l," but that a recovery from this inhibition was seen after about two weeks. In contrast, Basic Green 4 (at levels of 150 ppm) did not appear to have any significant effect upon anaerobic digestion of sewage sludge.

Michaels and Lewis (1985) tested the activity of gentian violet, at 0.5 or 5.0 mg/L (ppm) in agar plates, against field isolates of bacteria and algae. They found that the microbial survival rates were only 70% or 21% at these two concentrations. They conclude that gentian violet showed relatively high toxicity to aquatic bacteria at 5 ppm and that such toxicity could cause a reduction in the concentration of heterotrophic bacterial populations. In turn, this could decrease the normal mineralization of organic compounds, unless gentian violet was degraded by anaerobic microbes.

Gentian violet has been used as an antifungal agent in poultry feeds and has been determined to exhibit an overall antifungal activity greater than some other compounds used in this manner (Chen and Day, 1974; Hall and Hamilton, 1982; Stewart et al., 1977). Stewart et al. (1977) report crystal violet exhibiting mold inhibiting activity at levels $\geq$ 2 ppm (ug/ml).

Kropinski et al. (1978) examined the susceptibility of a parental strain and mutants of Pseudomonas aeruginosa to crystal violet. The minimum inhibitory concentration (MIC) for this dye was determined to be 0.625 mg/ml (625 ppm).

Kowalska (1984) tested a variety of antibacterials for activity against bacteria that cause bee brood diseases. Crystal violet was moderately effective in inhibiting these bacteria, with MIC's ranging from 80 to 300 ppm.

Gentian violet and its leuko metabolite have been found to be directly toxic to or mutagenic in a variety of microbial genetic toxicity test systems (Hass et al., 1986; Levin et al., 1982; Littlefield et al., 1985; Thomas and MacPhee, 1984). In fact, these test systems can be so "exceptionally sensitive to the toxic effects of crystal violet" that this compound is used as the standard diagnostic test for the presence of certain mutations in Ames strains of bacteria (Thomas and MacPhee, 1984).

b. Other single cell assays: The bioactivity of gentian violet extends to certain protozoan parasites found in human blood (trypanosomes). Docampo et al. (1983) report that gentian violet is widely used in blood banks in attempts to eliminate the transmission of Chagas' disease by blood containing the parasite Trypanosoma cruzi. Growth of a life stage of this parasite was substantially inhibited by 1.2 uM gentian violet (0.5 ppm) in the light. This parasite's growth was less inhibited by gentian violet treatments which were carried out in the dark.

Gentian violet can also cause cytotoxic effects in mammalian cell culture systems designed to look for genetic toxicity of test compounds (Au et al., 1978). These authors report that gentian violet is a mitotic poison, causes chromosome breakage, and they think that it should be considered a biohazardous substance because it caused severe cytogenetic toxicity, "among the highest of all compounds thus far tested."

c. Aquatic animals: Lio-Po and Sanvictores (1986) report on the testing of crystal violet as a fungicide to protect shrimp (Penaeus monodon) grown in artificial rearing systems. The sensitivities of various life stages of these shrimp to crystal violet were tested in a static bioassay system. Exposure of shrimp eggs or larvae to 1 ppm of crystal violet did not affect the egg hatching rate, however, none of the larvae survived beyond the first day of exposure.

Nishiuchi (1984) tested the toxicity of crystal violet and malachite green to several freshwater organisms. Both of these compounds demonstrated "strong toxicity" to fish, frogs, and other freshwater organisms with a consistent range of LC50's (concentration lethal to half of the organisms) of from 0.09 to 1.3 ppm.

This result was supported by test data on the toxicity of the crystal violet structural analogs, methyl violet and malachite green (Little and Lamb, 1973). These authors tested the static acute (96 hr) toxicity of 46 dyes to the fathead minnow. Methyl violet and malachite green were the two most toxic dyes tested, with LC50's = 0.05 ppm and 0.12 ppm, respectively.

Clarke and Anliker (1984) surveyed the available fish toxicity data on over 3,000 commercial dye products. Only 27 of the 3,000 compounds were found to result in LC50's <1 mg/L (ppm). Gentian violet, methyl violet, and malachite green are all structurally closely related and they all demonstrate acute toxicities to fish that are in this range. This group of triphenylmethane dyes therefore appears to be among the most acutely toxic to fish of all the dyes tested.

d. Laboratory mammals: EPA (1981) reports that gentian violet is moderately acutely toxic and lists the acute oral LD50's (dose lethal to half the organisms) for the mouse and the rat as = 405 mg/kg and 180 mg/kg, respectively. The lowest intraperitoneal dose that was fatal to the mouse, rat, and guinea pig ranged from 10 mg/kg to 20 mg/kg.

Littlefield et al. (1985) tested gentian violet for its chronic toxicity and carcinogenic effect in mice. The authors conclude that "gentian violet appears to be a carcinogen in mice at several different organ sites." Additional information concerning the carcinogenicity and mutagenicity of gentian violet is contained in FDA's proposal to declare gentian violet a food additive when added to animal feed.

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Appendix B: Environmental Assessment Data for Propionic Acid

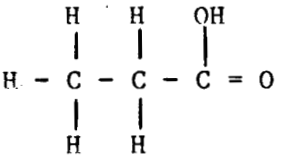
1. Chemical identity and properties.

   a. Common names: propionic acid (The Merck Index, 1976)
   propanoic acid (The Merck Index, 1976)
   methylacetic acid (The Merck Index, 1976)
   ethylformic acid (The Merck Index, 1976)
   carboxyethane (RTECS, 1982)

   b. Chemical names: Same as common names

   c. CAS registry number: 79-09-4 (RTECS, 1982)

   d. Structure:



   e. Empirical formula: $C_3H_6O_2$ (The Merck Index, 1976)

   f. Molecular weight: 74.08 (The Merck Index, 1976)

   g. Melting point: - 22°C (Verschueren, 1977)

   h. Boiling point: 141°C (Verschueren, 1977)

   i. Specific gravity: 0.992 (Verschueren, 1977)

   j. Flash point, Cleveland Open Cup, °F: 150 (Kirk-Othmer, 1968)

   k. Physical description: A water-white liquid with a sharp, irritating odor resembling strong vinegar (Kirk-Othmer, 1968)

2. Introduction into the environment through manufacturing.

The 1984 United States production of propionic acid for all uses was reported to be 95,867,000 pounds, of which 78,817,000 pounds were sold at 22 cents per pound, for a total value of $17,020,000 (USITC, 1985).

Formerly, the principal source of propionic acid was the liquid condensate from the manufacture of charcoal. The oxo process is the major method of manufacture, involving the reaction of ethylene and carbon monoxide under reductive conditions. Large quantities of the acid are also obtained from liquid-phase oxidation of propane, butane, mixed paraffins and paraffin wax. A further process is the direct oxidation of 1-propanol with nitric acid (Kirk-Othmer, 1968).

Propionic acid and its salts find uses in the production of plastics, fibers, mold inhibition in human and animal feeds, and in the production of various herbicides, drugs, perfumes and flavors (Kirk-Othmer, 1968).

Neither OSHA nor NIOSH has any exposure standard or criteria for propionic acid. ACGIH has established a Threshold Limit Value of 10 ppm (30 mg/m^3), which is largely based on analogy with acetic acid and is intended to prevent undue irritation to the eyes and respiratory passages. Symptoms of over-exposure from inhalation include sore throat, coughing and shortness of breath; from eye contact redness, pain and blurred vision; from skin absorption redness and pain (NIOSH, 1982).

It is documented that propionic acid is a component of domestic sewage and of effluent from wastewater treatment facilities. Verschueren (1977) lists propionic acid as present in domestic (untreated) sewage at 1.2 to 8 mg/L (ppm) and that the average content in secondary sewage effluent (a biological treatment process) is 13.7 ppb. Gossett et al. (1983) measured quarterly the concentration of propionic acid in effluent from the Los Angeles County wastewater treatment facility from November 1980 to August 1981, finding an average of 20 ppb. The source of propionic acid in these wastes was not identified. Trudell et al. (1985) found that high strength acidic wastewater containing 1,000 ppm propionic acid and 500 ppm acetic acid could be successfully treated anaerobically. Lin et al. (1986) were successful in treating even higher concentrations of mixed low molecular weight volatile fatty acids (including a high proportion of propionic acid).

No details could be located on the amounts and types of wastes that might enter the environment or the workplace as a consequence of the production of propionic acid or its addition to feeds as a fungistatic agent. Based on the relatively high vapor pressure and low odor threshold (cited between 34 ppb and 20 ppm, see Volatility, below), propionic acid would appear to be more of an odor control problem during production, as workers would be able to detect its presence before or about the same time the 10 ppm TLV was reached in the workplace. Prolonged exposure would be expected to cause eye and mucous membrane irritation.

3. Introductions into the environment through use in animal feeds.

Propionic acid and propionic acid salts (such as calcium propionate) are added to poultry feeds as fungistatic agents. Dilworth et al. (1979) tested a number of fungistatic agents, including calcium propionate at 0.1% in poultry feeds with moisture levels up to 15%, and found that there were no significant effects on broiler performance at this concentration. In a related experiment, Chen et al. (1979) found that calcium propionate at 0.1% prevented increases in mold and yeast counts and prevented caking in poultry feeds with moisture levels up to 15% for a 4-week period of storage. Bacterial counts increased during this period, however. Tabib et al. (1984) list several factors,

including the concentration of limestone, fat, proteinaceous material high in basic amino acids, and corn variety, that appear to affect the apparent effectiveness of organic acids as mold inhibitors in poultry feeds.

Propionic acid is a naturally occurring component of animal wastes, particularly wastes that have decomposed anaerobically. Anaerobic microorganisms present in animal manure, human sewage and sewage sludge, industrial wastewaters, soils, ensilage and the rumens of ruminant animals produce as a metabolic end-product carboxylic volatile fatty acids, including acetic, propionic, butanoic, and higher molecular weight fatty acids. In anaerobic wastes, this production of acids by microorganisms is referred to as "acidogenesis." Anaerobic hydrogenic bacteria utilize propionic acid as an energy substrate, converting it to acetic acid. Acetic acid is then utilized by methanogenic anaerobes as an energy substrate, producing methane as a metabolic by-product (Bolte et al., 1986). Fatty acids are also utilized under oxidative (aerobic) conditions by microorganisms and higher animals as an energy source, thereby completing the recycling of carbonaceous matter from metabolic wastes to components of living cells.

Yasuhara et al. (1984) found propionic acid present in fresh swine feces and urine and rotted mixtures at 31.6, 0.43, and 13.7 ppm, respectively.

While the concentration of propionic acid present in wastes and bedding from poultry fed treated feed could not be found, from the above it could be concluded that any amount would not be particularly serious as an environmental impact threat. Some of the fed propionic acid would be expected to be metabolized by the poultry and their intestinal flora as an energy source, some of the excreted propionic acid would be expected to volatilize into the poultry house atmosphere along with other compounds such as ammonia, a large portion of any excreted propionic acid would be expected to be utilized by aerobic and anaerobic bacteria present in the litter. Any remaining propionic acid would be expected to be utilized readily at the time the waste was incorporated into agricultural soils by the microflora present.

4. Fate of propionic acid in the environment.

   a. Solubility: Freely soluble in water (Kirk-Othmer, 1968)

   b. Octanol/water partition coefficient: log Kow = 0.33 (Gossett, et al., 1983)

   c. Dissociation constant: 1.34×10^{-5}, pK = 4.87 at 20°C (CRC, 1986)

   d. Adsorption/desorption: No sorption coefficient could be found, however, based on its ability to dissociate at soil pH levels, some sorption of the dissociated form to cations, such as calcium and aluminum, present in soils might be expected.

e. Volatility: Hoshika (1981) detected propionic acid in ambient air near accumulated poultry manure and in exhaust air from a poultry manure dryer at 82 and 1,190 ppb, respectively. The air in a pig pen was found to have 990 ppb propionic acid. While propionic acid was not found to be the source of the characteristic malodor in manure, its detection limit by humans is low. Hoshika (1981) cites the odor recognition threshold for propionic acid to be 34 ppb. Guenzi (1981) cites a much higher odor threshold of 20 ppm (v/v). The vapor pressure of propionic acid is reported by Verschueren (1977) as 2.9 mm Hg at 20°C.

f. Biodegradation: Guenzi (1981) experimented in laboratory systems with cattle manure under various redox conditions, i.e., controlled presence of oxygen. It was found that an initial propionic acid concentration of 400 mg/Kg of manure, when added to 2 liters of water, was no longer detectable within 7 days under aerobic conditions. The authors concluded that the propionic acid, including any that was formed under these conditions, was metabolized by the aerobic microorganisms present. As the redox potential was lowered, i.e., oxygen was depleted in the mixture, aerobic organisms disappeared and facultative and obligate anaerobes became predominant by day 29 of the experiment. At that time, propionic acid reappeared in the test system and continued to increase. On day 45 of the experiment, the propionic acid concentration was found to be 2.30 mg/Kg of the manure that was originally added to the test flask.

Urano and Kato (1986) ranked 78 organic compounds, including propionic acid, for biodegradability in aqueous solutions containing 100 ppm of the test compound. Propionic acid was found to biodegrade in 100 hours under the test system employed; the same time found for glucose. Propionic acid was ranked as readily biodegradable; beta-oxidation and the TCA (Krebs) cycle were identified as the probable biodegradation mechanisms used by the microorganisms present.

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Appendix C: Environmental Assessment Data for Benzoic Acid

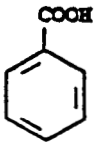
1. Chemical identity and properties.

   a. Common names: benzoic acid (The Merck Index, 1983)
      phenylformic acid (The Merck Index, 1983)
      dracyclic acid (The Merck Index, 1983)

   b. Chemical names: benzenecarboxylic acid (The Merck Index, 1983)

   c. CAS registry number: 65-85-0 (USAN, 1984)

   d. Structure (The Merck Index, 1983):



   e. Empirical formula: $C_7H_6O_2$ (The Merck Index, 1983)

   f. Molecular weight: 122.12 (The Merck Index, 1983)

   g. Melting point: 122.4°C (The Merck Index, 1983)

   h. Specific gravity: 1.27 (Verschueren, 1977)

   i. Vapor density: 4.21 (relative vapor density-air) (Verschueren, 1977)

   j. Boiling point: 249.2°C at 760 mm Hg (The Merck Index, 1983)

   k. Chemical reactivity: benzoic acid shows few exceptional chemical properties; normal carboxyl group reactions, predictable ring substitutions (Williams, 1985).

2. Introduction into the environment through manufacturing.

The Chemical Marketing Reporter (1984) provides the following information concerning the production of benzoic acid in the United States:

Producers	Capacity (millions lbs/yr)
Kalama, Kalama WA	140
Pfizer, Terre Haute, IN	10
Velsicol, Chattanooga, TN	60
Total	210

The reported demand for 1983 was 154 million pounds and for 1984, 165 million pounds (Chemical Marketing Reporter, 1984). A projected demand of 179 million pounds is also reported for 1988 (Chemical Marketing Reporter, 1984). Historical growth of the production for the years 1974-1983 has been a minus 0.5% per year; future growth is projected at 2% per year through 1988 (Chemical Marketing Reporter, 1984).

Benzoic acid is manufactured in the U.S. by liquid-phase oxidation of toluene employing cobalt catalysts (Williams, 1985). Under current air-emission standards it is likely that most manufacturers are using adsorption on activated carbon to treat vent gases (Williams, 1985).

Conversion of benzoic acid into plasticizers and benzoyl chloride consume most of the benzoic acid produced, although the production of sodium benzoate continues to be a major market for benzoic acid because it is such an important preservative in food products (Williams, 1985). Benzoic acid and its salts and esters are used in medicinals, veterinary medicines, food and industrial preservatives, cosmetics, resin preparations, plasticizers, dyestuffs, synthetic fibers, and intermediates (Williams, 1985). Sodium benzoate has been used for corrosion inhibition and to improve the properties of various alkyd resin coating formulations to improve gloss, adhesion, hardness, and chemical resistance. Primary uses of benzoic acid production reported in the Chemical Marketing Reporter (1984) are 54% for phenol, 18 percent for plasticizers, 13% for benzoyl chloride, 8% for sodium benzoate and 7% for other uses.

In one monitoring study, Gossett et al. (1983) identified 101 organic compounds in Los Angeles County wastewater treatment effluents collected quarterly from November 1980-August 1981. Concentrations of identified compounds ranged from 0.003 to 2,500 ug/l. Eleven compounds were present at greater than 100 ug/l including benzoic acid which was present at 400 ug/l (Gossett et al., 1983). The source of the benzoic acid in the waste effluent was not identified.

In 50 air samples from an industrial environment taken over a one year period yielded results ranging from none detected to 0.30 ppm benzoic acid (Halvorson, 1984).

No details could be found on the amounts and types of wastes that might enter the environment as a consequence of the production of benzoic acid for use as a mold inhibitor in poultry feed.

3. Natural introduction into the environment.

Benzoic acid is widely distributed in nature in free and combined forms (The Merck Index, 1983; Verschueren, 1977; Williams, 1985). Benzoic acid is present in a variety of plants (Altman and Dittmer, 1973). Gum benzoin (from styrax benzoin) may contain as much as 20% benzoic acid (The Merck Index, 1983; Williams, 1985). Acaroid resin (from Xanthorrhoca hasilis) contains 4.5-7% benzoic acid (Williams, 1985).

Benzoic acid is present in natural foods, particularly fruits and berries (Principles, 1978). Benzoic acid is also produced by a number of arthropod species (Altman and Dittmer, 1973). Smaller amounts of free acid are found in natural products of diverse character, e.g., scent glands of beaver, bark of wild blackcherry tree, cranberries, prunes, ripe clove and oil of anise seeds (Verschueren, 1977; Williams, 1985). It is also a natural excretion product of vertebrates including man, who normally excretes 0.7 g benzoic acid per day (Bridges et al., 1970; Principles, 1978). In a recent study of the biotransformation of benzoic acid in man (Sioufi and Pommier, 1982), controls excreted 2.16 mg of free benzoic acid and 470.7 mg of total benzoic acid per day. Huckle et al. (1981) report that benzoic acid showed little metabolic variation among the nine mammalian species tested and that urine is the primary route of excretion.

In man, benzoic acid facilitates the metabolic formation of hippuric acid (Figure 1) through the activation of CoA and reaction with glycine (Principles, 1978). In birds, benzoic acid is activated similarly but then reacts with amino groups of orthine to form ornithuric acid (Figure 2; Principles, 1978). Bridges et al. (1970) in studying the fate of orally administered benzoic acid in various species found all of the species examined excreted benzoic acid mainly as its conjugate, suggesting that the compound is not destroyed in these animals. They report that 5 female Sussex chickens excreted 56% of an orally administered dose of benzoic acid within 24 hours and that the metabolites excreted consisted of 54% ornithuric acid, 22% benzoic acid, 21% hippuric acid, and 3% benzoyl glucuronide (Bridges et al., 1970).

Figure 1. Structure of hippuric acid (Principles, 1978).

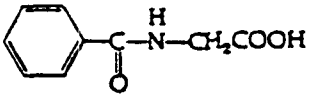
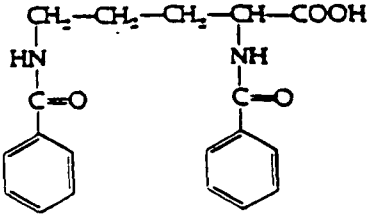


Figure 2. Structure of ornithuric acid (Principles, 1978).



Benzoic acid has also been found in soil humic material (Sato et al., 1985) and in runoff from decomposing crop residues (Glenn and Williams, 1985).

4. Introduction of benzoic acid through use in poultry feed.

There is no information available concerning the proportion of the total production of benzoic acid which may be used in poultry feed. FDA regulations (21 CFR 582.3021) provide for the GRAS use of benzoic acid at a level not to exceed 0.1 percent. Therefore, each ton of poultry feed could contain as much as 2 pounds of benzoic acid. Based on information provided in the environmental assessment for gentian violet, up to 8.4 million tons of poultry feed could be treated with a replacement product. Therefore, a maximum of 16.8 million pounds of benzoic acid could be used as a replacement for gentian violet as a mold inhibitor. This amount of benzoic acid represents only 8% of the total benzoic acid production in the U.S. (Chemical Marketing Reporter, 1984).

5. Fate of benzoic acid in the environment.

a. Solubility: The solubility of benzoic acid increases with both temperature and alkalinity (The Merck Index, 1983). The effect of temperature is displayed in Table 1.

Table 1. Solubility of benzoic acid in relation to temperature (The Merck Index, 1983).

Temperature (°C)	Solubility (g/l)
0	1.7
10	2.1
30	4.2
50	9.5
70	17.7
90	45.5
95	68.0

b. Octanol/water partition coefficient: log Kow = 2.03 (Gossett et al., 1983).

c. Dissociation constant: The pKa of benzoic acid is reported to be 4.2 (Principles, 1978), although Rubino and Berryhill (1986) reported that the pKa increases between a range of 4.17-5.66 depending upon the volume fraction of the cosolvent-water mixture. The Merck Index (1983) reports that a standard solution of benzoic acid has a pH of 2.8 at 28°C.

d. Adsorption/desorption: The adsorption of benzoic acid on six of ten soils tested by Lokke (1984) are provided in Table 2.

Table 2. Soil characteristics and Freundlich adsorption constants (Ka) and Koc for benzoic acid at 6°C (Lokke, 1984).

	soil pH	OC[1]	Clay[2]	Silt[2]	CEC[3]	K_a	K_{oc}
1	3.23	1.41	3	6	48	1.17	83
3	4.07	1.82	5	3	70	1.39	76
4	3.88	5.11	4	5	130	1.53	30
5	4.95	0.09	3	3	16	0.45	500
6	4.21	0.15	2	1	13	0.32	210
10	7.64	0.13	41	34	405	0	0

1 % organic carbon
2 % of soil
3 cation exchange capacity (meq/kg)

Variance of the Ka was significantly correlated with soil organic carbon ($P<0.006$), while other factors tested (pH, CEC and temperature) were not significant (Lokke, 1984). Of particular interest is that of the initial benzoic acid concentration of 10 mg/l, there were no irreversibly bound amounts of benzoic acid found (<1% of initial amount) (Lokke, 1984).

e. Biodegradation: Considerable information is available concerning the biodegradation of benzoic acid (Verschueren, 1977; DiGeronimo et al., 1979; Urano and Kato, 1986; Shelton and Tiedje, 1984; Banerjee et al., 1984; Freitag et al., 1985). DiGeronimo et al. (1979) reported that of 100 ug/ml benzoic acid incorporated into an activated sludge system, 100% of the compound degraded in less than 2 days. Verschueren (1977) reported a theoretical oxygen demand for benzoic acid of 1.96 and 5, 10 and 20 day BODs (biological oxygen demands) of 1.34, 1.40 and 1.45 by standard dilution technique with sewage seed material. Urano and Kato (1986) ranked benzoic acid as degradable as sugars, aliphatic compounds, amino acids, carboxylic acids, and linear alcohols and aldehydes using an electronic respirometer technique to determine relative biodegradability. In a preliminary study, Shelton and Tiedje (1984) determined that benzoic acid was mineralized to greater than 75% of theoretical methane production in 8 weeks under anaerobic conditions in 10% secondary sludge. Banerjee et al. (1984) studied pure and mixed cultures of microorganisms and natural waters from various sources and found that benzoic acid was not only rapidly degraded in all samples tested, but that it acted as a sole nutrient source supporting the growth of microbiol organisms. In one sample benzoic acid was one of only two compounds degraded (Banerjee et al., 1984). Freitag et al. (1985) report that 65.4% of applied benzoic acid was degraded by activated sludge.

Benzoic acid is also noted to be utilized by plants (Carew and Bainbridge, 1976). Tissue cultures of Catharanthus roseus, Apocynum cannabinum, and Conium maculatum grown in medium

with a concentration of 12.5 mg benzoic acid per 50 ml of medium caused a 4- and 7-hydroxylation of benzoic acid to 4-OH benzoic acid after 1 day of incubation following substrate addition (Carew and Bainbridge, 1976).

f. Photodegradation: Benzoic acid appears to be only slightly susceptible to photodegradation based on a study reported by Freitag et al. (1985) in which only 10.2% of the benzoic acid used in the test was degraded in light with wavelengths greater than 290 nm.

h. Bioaccumulation: Freitag et al. (1985) utilizing 14C-labelled organic compounds determined the bioaccumulation factors (BF) for benzoic acid and other chemicals in sludge, algae, and fish, and retention in rats. They report BFs for benzoic acid of 1,300 for activated sludge, <10 for algae (Chorella fusca), and <10 for fish (golden ide). Rats retained only 0.1% of the applied dose (Freitag et al., 1985). Similarly, Geyer et al. (1984) reported a BF in Chorella fusca of three.

6. Effects of benzoic acid in the environment.

A considerable amount of information on the effects of benzoic acid on environmental organisms is available. Some of the data reviewed for this assessment include: Chou and Patrick, 1986; Eklund, 1985; Hartenstein, 1982; and Schafer and Bowles, 1985. Of relevance is that Gossett et al. (1983) report that benzoic acid is not on EPA's Priority Pollutant list of 1977, even though it is relatively prevalent in the environment. Additionally, Halfon et al. (1986) in a formal approach to rank chemicals for environmental hazard according to relevant fate and effects data ranked benzoic acid as one of the 9 least hazardous chemicals out of the 34 chemicals evaluated. The following are some of the quantifiable biological effects of benzoic acid:

Inhibition of cell multiplication (Verschueren, 1977)
Pseudomonas putida 480 mg/l
Microcystis aeroginosa (algae) 55 mg/l

MICs (minimum inhibitory concentration; mmol/l) for undissociated and dissociated benzoic acid (Eklund, 1985).

organism	undiss.	diss.
B. subtilis ATCC 6633	0.8	200
B. subtilis W23	0.6	180
B. cereus	0.5	120
E. coli	1.2	90
Ps. aeruginosa	9.5	140
S. aureus	2.2	190
C. albicans	0.8	90

TLm (Median tolerance limit = LC50) for mosquito fish (Verschueren, 1977):

24 hour = 240 mg/l
48 hour = 255 mg/l
96 hour = 180 mg/l

Rat acute oral LD50 1.7 g/kg (Verschueren, 1977)

In earthworms, benzoic acid was reported to be relatively innocuous and had significant effects on growth only at the highest concentration tested (1 g/g media). Benzoic acid had no significant effects on earthworm mortality (Hartenstein, 1982).

Benzoic acid is known to be a skin, eye, and mucous membrane irritant (The Merck Index, 1983). It is also an upper respiratory irritant (Halvorson, 1984). NIOSH (1982) indicates that benzoic acid is only a moderate skin irritant with a 6-hour intermittent irritant dose of 22 mg. Eye irritation in rabbits is reported as severe at a dose of 100 mg (NIOSH, 1982). No Threshold Limit Value (TLV) has been established for benzoic acid (Halvorson, 1984). However, Halvorson (1984) notes that benzoic acid's potential adverse occupational effects are noted on the Material Safety Data Sheets for benzoic acid and that the use of appropriate occupational safeguards to prevent these adverse effects could be expected to be in use at the manufacturing facilities.

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