CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 50-744

ENVIRONMENTAL ASSESSMENT AND/OR FONSI

3.4 Environmental Assessment

In the information following this page, the sponsor is providing an environmental assessment pursuant to 21 CFR 314.50(d)(1)(iii) and 21 CFR 25.31.

ENVIRONMENTAL ASSESSMENT

1. **Date:** November 3, 1995

2. **Applicant:** CollaGenex, Inc.

3. **Address:** 301 South State Street
Newtown, PA 18940

4. **Proposed Action:** This environmental assessment has been prepared as a required portion of a New Drug Application (NDA) for Periostat™ (doxycycline hyclate, 20 mg capsules USP). The proposed action requests U.S. Food and Drug Administration approval for Periostat as part of a professional oral health program to promote attachment gain and reduce pocket depth and bleeding on probing in patients with adult periodontitis.

The antimicrobial activity of doxycycline is believed to result from inhibition of bacterial protein synthesis at doses greater than two-fold the dose proposed in this NDA. In addition, doxycycline has been found to inhibit extracellular collagenase activity associated with adult periodontitis arising as a result of the neutrophil-derived host response to bacterial infection of the periodontal pocket.

Periostat will be used by individuals throughout the United States. The number of patients seeking or under treatment for periodontal disease in the U.S. is estimated at [redacted]. For this population the availability of effective therapy to complement standard treatments is clearly needed.

Doxycycline hyclate drug substance is manufactured and supplied by:

HOVIONE Macau - Sociedade Quimica, LDA.
[redacted]

HOVIONE Macau is located on Taipa Island of the Territory of Macau, one hour by hydrofoil from Hong Kong. The facility is situated on a flat site comprising [redacted] square meters near Taipa Village. The site is bordered on the north by a municipal road, on the south and east by a brushy hill, and on the west by a currently undeveloped lot comprising [redacted] square meters. The climate of this area is considered tropical.

Finished drug product (Periostat) is produced by:

Applied Analytical Industries, Inc. (AAI)
[redacted]

The AAI Clinical Services Division is located on a [redacted] site in a light industrial section of New Hanover county just inside the city limits of Wilmington, NC.

5. Chemical Substances that are the Subject of the Proposed Action:

Drug Substance:

HOVIOVE Macau considers the identity of chemical substances employed in the production of doxycycline hyclate to be confidential information not subject to public disclosure pursuant to the provisions of 21 CFR 25.30(b). However, both the applicant and HOVIONE Macau recognize the pertinence of this information to the environmental review of the proposed action. Therefore, for purposes of the required environmental review the applicant incorporates this information by reference to HOVIONE Macau's AADA [redacted]. A letter from HOVIONE Macau authorizing the Food and Drug

Administration to make reference to AADA [redacted] in support of the proposed action is provided in Appendix 1.

Finished Drug Product:

a. Active Drug Substance:

United States Adopted Name (USAN): Doxycycline Hyclate

British Approved Name (BAN): Doxycycline Hydrochloride

International Non-Proprietary Name (INN): Doxycycline Hydrochloride

C.A.S. Number: 24390-14-5

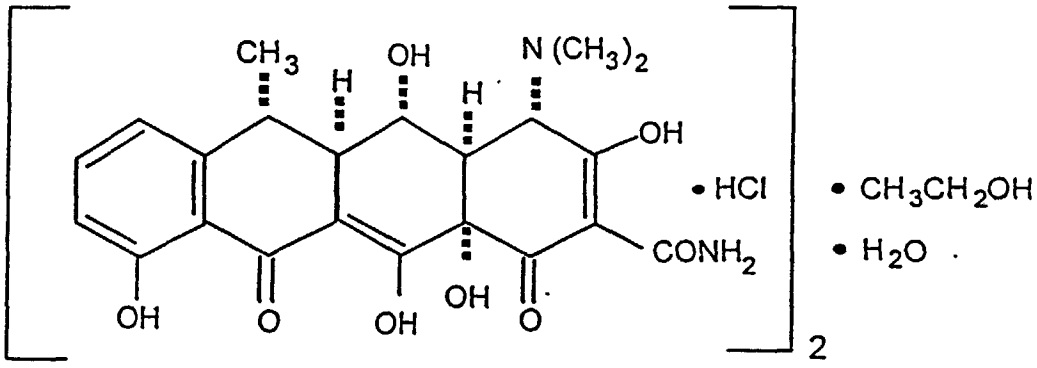
Chemical Names:

- Doxycycline hydrochloride hemihydrate hemi-ethanolate
- α-6-deoxy-5-hydroxytetracycline hydrochloride
- α-6-deoxyoxytetracycline hydrochloride
- 4-dimethylamino-1, 4, 4a ,5 ,5a, 6, 11, 12a-octa-hydro-3, 5, 10, 12, 12a-pentahydroxy-6α-methyl-1, 11-dioxonaphthacene-2-carboxamide, hydrochloride, hemihydrate, hemiethanolate

Chemical Formula: $(C_{22}H_{24}N_2O_8)_2 \bullet 2HCl \bullet H_2O \bullet C_2H_5OH$

Molecular Weight: 1025.89

Structural Formula:



Physical characteristics:

- A yellow, hygroscopic crystalline powder.
- Slightly ethanolic odor.
- Bitter taste.

Solubilities:

- Freely soluble in water and in methanol.
- Sparingly soluble in alcohol.
- Practically insoluble in chloroform and ether.
- Dissolves in aqueous solutions of alkali hydroxides and carbonates.

Impurities (Less than 2%):

- Methacycline
- 6-epidoxycycline

An MSDS for doxycycline hyclate is provided in Appendix 2.

b. Excipients:

1.) Microcrystalline cellulose, NF

CAS No. 9004-34-6

An odorless, white, free flowing powder; insoluble in water with a pH of 5.0 - 7.0 as an 11% solids dispersion; bulk density in 0.3 g/cc. It is a carbohydrate substance with a general chemical formula of $(C_6H_{10}O_5)_x$. MSDS for microcrystalline cellulose are provided in Appendix 3.

2.) Magnesium Stearate, NF

CAS No. 557-04-0

A fine white to yellow-white powder having a slight fatty acid odor; insoluble in water and ether, soluble in hot alcohol with a melting point of 150°-170°C (302°-338°F). It has a pure molecular weight of 591.2 and a chemical formula of $Mg(C_{18}H_{35}O_2)_2$. MSDS for magnesium stearate are provided in Appendix 4.

6. **Introduction of Substances into the Environment:** The sites of production are as provided in preceding section 4.

Drug Substance:

For production of doxycycline hyclate drug substance, HOVIONE Macau has its own solvent recovery plant and waste water treatment plant for minimizing introduction of substances into the environment. Solid wastes are incinerated in the municipal incinerator located in Taipa. With regard to emission of substances to the environment, HOVIONE Macau has provided a letter issued by the Macau Economic Department certifying that the HOVIONE site operates its waste handling and disposal procedures in compliance with the relevent Macau Industrial Legislation. This letter, with official translation, is provided in the following pages in lieu of supplying the complete information listed in this section.

Drug Product:

The following substances may be emitted [redacted] during production of the subject drug product:

1. Doxycycline hyclate
2. Microcrystalline cellulose
3. Magnesium stearate
4. Hard gelatin capsules
5. Water
6. Isopropyl alcohol
7. Methyl alcohol
8. Dipropylene glycol methyl ether (alkaline liquid cleaner: Rapid Kleen®)

[redacted] AAI [redacted] used for the manufacture and packaging of this product. [redacted] rooms are equipped with [redacted] HVAC system. The exhaust side of the HVAC system consists of a bank [redacted] for collection of any particulate matter produced. The exhaust air is [redacted] passed through [redacted] HEPA filters [redacted] before being released to the atmosphere. Any dust not collected by the air filtration system is vacuumed, swept or mopped up daily. [redacted], and [redacted] are used in cleaning the production equipment and the walls and floors of the Production and Packaging Rooms.

All other air emissions also meet the limitations of the Federal EPA and the State of North Carolina prior to their ventilation to the atmosphere. If raw material dust particles collect on the exhaust air filters in the manufacturing area during the weighing and charging of the raw materials, these filters are cleaned or replaced and the residue disposed of in accordance with appropriate hazardous waste requirements.

Pharmaceutical solid wastes from the manufacturing and testing processes consist primarily of [redacted], [redacted] and [redacted]. This material is disposed of by incineration [redacted] under the supervision of an AAI employee.

The estimated yearly amount of solid recoverable and unrecoverable waste associated with the manufacture of this drug product is shown in Table 1.

Process wastewater, which consists primarily of manufacturing and testing equipment rinsing, is discharged to the [redacted] in [redacted] and the [redacted]. The amount of unrecoverable pharmaceutical excipients and active drug substances discharged yearly by this means is minimal.

Table 1

Estimated Yearly Recoverable and Unrecoverable Pharmaceutical Loss Associated with the Manufacture of Periostat Capsules

Estimated Total Yearly Recoverable Loss:

Doxycycline hyclate: [redacted]
Microcrystalline Cellulose: [redacted]
Magnesium Stearate: [redacted]
Total: [redacted] ([redacted])
Plus: [redacted] hard gelatin capsules ([redacted])

Estimated Total Yearly Unrecoverable Loss:

Doxycycline hyclate: [redacted]
Microcrystalline Cellulose: [redacted]
Magnesium Stearate: [redacted]
Total: [redacted] ([redacted])

Recoverable losses are collected and disposed of by means of an EPA-approved incinerator. Unrecoverable losses are due primarily to product particulates [redacted], and [redacted] during processing and clean-up. These articles are collected and treated as recoverable waste for disposal. A minimal amount of unrecoverable loss occurs [redacted].

The occupational exposure of workers during manufacturing and the testing of material [redacted] is historically low. Plant personnel have been instructed in safe product handling practices and standard operating procedures and policies are in place to assure compliance. Appropriate safety equipment [redacted] are worn during manufacturing and the testing of materials. AAI has certified its compliance with applicable OSHA safety and health standards promulgated by 29 CFR 1900 (Appendix 5).

Wastage generated by operations at AAI pursuant to approval of the proposed action is to be treated and handled [redacted]. AAI has attested to its compliance with applicable emissions requirements [redacted]

Recoverable losses are collected and disposed of [redacted]

Unrecoverable losses are due primarily to product particulates [redacted]. These [redacted] are collected and treated as recoverable waste for disposal. A minimal amount of unrecoverable loss occurs [redacted]

The occupational exposure of workers during manufacturing and the testing [redacted] is historically low. Plant personnel have been instructed in safe product handling practices and standard operating procedures and policies are in place to assure compliance. Appropriate safety equipment [redacted] are worn during manufacturing and the testing of materials. AAI has certified its compliance with applicable OSHA safety and health standards promulgated by 21 CFR 1900 [redacted].

Wastage generated by operations at AAI pursuant to approval of the proposed action is to be treated and handled by [redacted] with little or no discernible impact of the resulting waste streams on current emissions permit levels. AAI has attested to its compliance with applicable emissions requirements in the statement provided hereafter.

7. **Fate of Emitted Substances in the Environment:**

As stated in the previous section, a minimal amount of doxycycline hyclate is irretrievably lost [redacted] during manufacture of the drug product [redacted]. Essentially all discharge of doxycycline hyclate as a result of patient use of the drug product will also be into wastewater treatment plants. Therefore, consideration of the environmental fate of discharged doxycycline hyclate is given solely to the aquatic compartment.

The average annual estimate of doxycycline hyclate involved in producing Periostat Capsules pursuant to approval of the proposed action is placed at [redacted]. Assuming a total manufacturing loss [redacted] of [redacted]% ([redacted]) and discounting the amount of loss [redacted] as negligible, a total of [redacted] of doxycycline hyclate would be expected to enter wastewater treatment plants via discharge through patient use. In a worst case situation wherein no degradation of doxycycline hyclate is assumed to occur during wastewater treatment, [redacted] of the drug substance would be expected to enter the aquatic environment subsequent to discharge from wastewater treatment plants.

Environmental effects parameters were evaluated according to the Environmental Assessment Technical Test Matrix based upon criteria established from the scientific literature [redacted]. The matrix comprises four tiers: Tier 0, which provides guidance as to which subsequent tiers are most likely to be affected by the target compound; Tier 1, representing the aquatic compartment; and Tiers 2 and 3 which represent the terrestrial and atmospheric compartments, respectively.

Tier 0 Criteria

Tier 0 evaluations for the target compound include water solubility, hydrolysis rate, vapor pressure estimate, dissociation constant(s), and octanol/water partition coefficient (Log P).

Water Solubility: [redacted] one gram doxycycline hyclate is soluble in three milliliters water. This equates to a solubility of 333, 333 mg/L = 333,333 ppm (~33%).

Hydrolysis Rate: The hydrolysis rate for doxycycline is unknown and for purposes of this assessment it is assumed that little or no hydrolysis occurs.

Dissociation Constants: Wiebe and Moore [redacted] reported pKa values of 3.4, 7.7, and 9.7 for pKa 1 - 3, respectively. These values are very similar to values reported for other tetracyclines [redacted].

Octanol/Water Partition Coefficient (Log P): According to Cooke and Gonda (ref. 3), the apparent partition coefficient for doxycycline hydrochloride (The British Approved Name for doxycycline hyclate) at 25°C is 0.60, yielding a Log P value of -0.2218 (similar to tetracycline, for which the Log P value is -1.05).

Vapor Pressure: The vapor pressure of doxycycline hyclate is unknown; however, due to its crystalline structure and relatively stable nature, the vapor pressure is expected to be small (less than $10E^{-7}$ torr).

The preceding values are applied to the Tier 0 criteria as follows: if water solubility is greater than $10E^{-5}$ (10,000 ppm), Log P is less than 2, and estimated vapor pressure is

less than $10E^{-7}$ torr, then doxycycline hyclate would most likely be found in the aquatic compartment (Tier 1) and further evaluations need only be done according to those criteria. Doxycycline hyclate complies with these Tier 0 criteria; thus, further evaluations are performed only according to Tier 1 criteria.

Tier 1 Criteria

Microbial Toxicity: The microbial toxicity of doxycycline, a synthetic derivative of oxytetracycline, in the aquatic environment is unknown. However, oxytetracycline itself is approved by the Food and Drug Administration's Center for Veterinary Medicine for use in treating certain diseases of fish and lobsters. Therefore, the use of oxytetracycline is beneficial in controlling diseases in the aquatic environment and does not pose adverse effects on this compartment [redacted]. Due to doxycycline's similarity to its parent compound oxytetracycline, no appreciable microbial toxicity for doxycycline in the aquatic compartment is expected.

Aerobic or Anaerobic Biodegradation: The amenability of doxycycline to either biodegradation process is not known and for purposes of this assessment it is assumed little or none occurs.

Acute Aquatic Toxicity: The specific toxicity of doxycycline to aquatic organisms is not known. However, as previously described, its parent compound, oxytetracycline, has been determined to pose no adverse aquatic effects, and tetracyclines (in general) are not acutely toxic to fish in concentrations around 10 ppm in water [redacted]. Doxycycline is therefore expected to be similarly nontoxic to fish.

At this point in the Tier 1 matrix the maximum expected emitted concentration (MEEC) is calculated. The MEEC is a measurement, in ppm, of doxycycline hyclate to be

expected in the aquatic environment. The formula for performing this calculation is as follows:

MEEC (ppm in environment) = lbs./year production x $8.9E^{-9}$

where $8.9E^{-9}$ is the product of:

(yr/365 days) (day person/150 gallons) (1/246 million persons)

(gallons/8.34 lb.) (one million)

As stated earlier in this section, [redacted] of doxycycline hyclate is expected to be produced yearly (less [redacted] production loss), which equates to [redacted] lbs. Entering this factor into the formula above yields:

MEEC (ppm in environment) = [redacted] lbs. x $8.9E^{-9}$

= [redacted]

A value for EEC (expected environmental concentration) is then obtained by subtracting values for aquatic biodegradation processes from the MEEC value. Since no such processes are known or assumed to occur for doxycycline hyclate, the EEC and the MEEC are the same (i.e., 0.0000[redacted] ppm).

The EEC value is then compared with the acute aquatic toxicity (LC_{50}) for doxycycline hyclate and, if this EEC value is less than 1% of the toxicity measure, then further compartmental evaluations need not be made.

As stated previously, the acute aquatic toxicity of doxycycline is not known; however, concentrations of 10 ppm of other tetracyclines are not toxic to fish. If this nontoxic concentration for other tetracyclines is taken as the LC_{50} value for doxycycline, then 1% of this value equals 0.1 ppm. If this concentration is compared to the EEC value, it is clear that the EEC is the vastly lesser of the two values (EEC = 0.000[redacted] ppm; 1% LC_{50} = 0.1 ppm); thus, further compartmental evaluations are not made.

8. **Environmental Effects of Released Substances:** Pursuant to the evaluations provided in section 7, it may be reasonably concluded that no significant environmental effects would be expected due to the presence of 0.000[redacted] ppm doxycycline hyclate.

9. **Use of Resources and Energy:** AAI, the drug product manufacturer, obtains its potable water supply from [redacted] in [redacted] and the [redacted]. The water demand for AAI's manufacturing and testing activities related to the proposed action, including [redacted], is estimated at less than [redacted]. Therefore, no significant environmental impact or depletion of water resources is anticipated.

The testing and production operations at AAI rely almost totally on [redacted] which is obtained from [redacted], and [redacted]. Therefore, the level of AAI's [redacted] consumption related to the proposed action is not expected to have a significant impact on the geographical region's total supply of this resource.

Since AAI's testing and production operations rely almost totally on [redacted]; depletion of fuel resources is not expected.

Operations consistent with the proposed action are conducted within AAI's existing facilities [redacted].

No impact on endangered species or any place listed, or eligible for listing, in the National Register of Historic Places is anticipated [redacted]

The equipment utilized in AAI's manufacturing and testing operations relating to the proposed action is not expected to produce noise sufficient to adversely impact the environment.

Since wastes generated from operations [redacted] are treated or handled by existing systems with little or no measurable impact on these systems, the resources and energy required to dispose of the wastes from the proposed action are considered minimal or immeasurable.

10. **Mitigation Measures:** Since there is no substantial additive environmental impact, existing environmental pollution control systems [redacted] are believed to be capable of dealing with measures necessary to avoid any potential adverse environmental impacts. The facilities operate in accordance with Spill Prevention and Countermeasure Plans, which relate to the handling and storage of chemicals and wastes for ultimate disposal. AAI employs normal preventive maintenance measures to avoid equipment failures. Any increase in manufacturing and testing operations due to the proposed action is not expected to significantly add to the existing environmental burden.

11. **Alternatives to the Proposed Action:** No significant adverse environmental impact is anticipated; therefore, alternatives to the proposed action are not considered.

12. **List of Preparers:**

[redacted]
Shotwell & Carr, Inc.
Dallas, Texas 75234

[redacted]
Shotwell & Carr, Inc.
Dallas, Texas 75234

[redacted]
AAI Clinical Services Division
Wilmington, NC 28405

[redacted]

13. The undersigned official certifies that the information presented is true, accurate and complete to the best of the knowledge of the firm responsible for preparation of the environmental assessment:

Christopher Powala
Director, Drug Development and Regulatory Affairs
CollaGenex, Inc.

14. References:

1. Interim Guidance to the Pharmaceutical Industry for Environmental Assessment Compliance Requirements for the FDA. Pharmaceutical Manufacturers Association. July 1991.
2. Wiebe, J.A. and Moore, D.G. (1977). Oxidation photo-sensitized by tetracyclines. J. Pharm. Sci. 66, 186-189.
3. Cooke, D.T. and Gonda, I. (1971). Temperature and concentration dependent partitioning of three tetracyclines between phosphate buffers and octanol. J. Pharm. Pharmac. 29, 190-191.
4. Environmental Assessment for National Academy of Sciences/National Research Council, Drug Efficacy Study Group. Finalization for Oxytetracycline Water Soluble and Premix Formulations for Food Producing Animals. Bureau of Veterinary Medicine Environmental Impact Staff. October 1983.
5. Draft Environmental Impact Statement, Subtherapeutic Antibacterial Agents in Animal Feeds. Bureau of Veterinary Medicine, Food and Drug Administration. July 1978.

15. Appendices:

[redacted]

[redacted]

MATERIAL SAFETY DATA SHEET FOR DOXYCYCLINE HYCLATE

4-0228

[redacted]

DOXYCYCLINE HYCLATE
MATERIAL SAFETY DATA SHEET

COMMERCIAL PRODUCT NAME

Doxycycline Hyclate

1. CHEMICAL CHARACTERISATION

1.1	Chemical name:	2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride hemiethanolate hemihydrate, [4S-(4α,4aα,5α,5aα,6α,12aα)]
1.2	Chemical formula:	$C_{22}H_{24}N_2O_8.CH_5ClO$
1.3	CAS number:	24390-14-5
1.4	Chemical family:	Semi-synthetic tetracycline antibiotic
1.5	Description:	Yellow crystalline powder

2. PHYSICAL AND SAFETY DATA

2.1	Change in physical state:	Not relevant
2.2	Bulk density:	About 0.3 to 0.6 g/cm^3 (before compacting), according to crystal si
2.3	Vapour pressure:	Not applicable
2.4	Viscosity:	Not applicable

[redacted]

2.5	Solubility: - Water - Methanol	 Soluble (1 g/3 ml) Soluble (1 g/4 ml).
2.6	pH:	2.0 to 3.0 (1% sol. in water)
2.7	Flash point:	Not applicable
2.8	Ignition temperature:	Not applicable
2.9	Explosion limits:	Not applicable
2.10	Thermal decomposition:	Carbonizes at 240°C
2.11	Hazardous decomposition products:	Emits toxic fumes when heated to decomposition
2.12	Hazardous reactions:	
	- Incompatibility with other substances	Not applicable
	- Risk of bursting	Not applicable
	- Spontaneous polymerisation	Does not occur
	- Risk of dust explosion	This substance is assumed to be combustible. As with all dry powders, it is advisable to ground mechanical equipment in contact with it to dissipate any potential build-up of static electricity
	- Spontaneous ignition	Does not occur
2.11	Further information:	The substance is stable from a safety point of view. Protect fro. light.

[redacted]

3. TRANSPORT

3.1 Product must be transported at a cool temperature and protected from direct sunlight

4. REGULATIONS

On the basis of the data available, doxycycline hyclate is not a dangerous substance. However, the usual precautionary measures for handling chemicals should be observed.

5. PROTECTIVE MEASURES, STORAGE AND HANDLING

5.1 Protective measures:

- Storage	Keep the containers tightly closed in a dry cool place and away from direct sunlight or UV irradiation
- Handling	Open and handle the containers with care, in an adequately ventilated room and avoiding the formation of dust

5.2 Personal protective equipment:

- Respiratory protection:	When handling the product, use a dust repiratory mask.
- Hand protection:	Rubber gloves
- Eye protection:	Safety goggles
- Protective clothing and other equipment:	Overalls. Protect exposed skin

5.3 Industrial hygiene:

Contact with, or inhalation of dust should be avoided. Containers should be kept tightly closed and handled with adequate ventilation

[redacted]

5.4 Protection against fire and explosion:

This material is assumed to be combustible. Emits toxic fumes when heated. Use self-contained breathing apparatus during fi

5.5 Disposal:

Spilt product or waste should be disposed of according to loca legislation, preferably by incineration. Alternatively, produc could be returned to HOVIONE.

6. MEASURES IN CASE OF ACCIDENTS AND FIRES

6.1 After spillage:	Use dust respiratory mask and rubber gloves. Avoid dust. Wash contaminated clothing before re-use.
6.2 Extinguishing media:	Water spray, dry chemical, carbon dioxide or foam. Choice will depend on the nature of the surrounding fire

6.3 First aid:

Remove from exposure. Remove contaminated clothing. Persons developing hypersensitivity reactions must receive medical attention. For eyes or skin contact - flush affected area with water. Inhalation may cause irritation of respiratory tract - remove to fresh air. Ingestion may cause stomach irritation, nausea and vomiting. Obtain medical attention.

7. INFORMATION ON TOXICITY

7.1 Type of hazard to man:

- Threshold limit value: None established

[redacted]

7.1 Type of hazard to man (contd):

- Signs and symptoms of overexposure:	Possible allergic reaction to dus if inhaled or in contact with ski Stomach irritation, nausea and vomiting when ingested
- Acute toxicity effects:	Eyes, skin and/or respiratory tra irritation; nausea and vomiting
- Chronic toxicity:	Possible hypersensitisation of su ceptible persons
- Carcinogenicity:	Not known or suspected to be a carcinogen

7.2 Results from animal experiments:

- LD_{50} oral:	1900 mg/kg (mice)
	500 mg/kg (dogs)
- LD_{50} intravenous:	241 mg/kg (mice)
	>100 mg/kg (dogs)

7.3 Other information:

- As a general rule, avoid contact with, and inhalation of dust when handling doxycycline hyclate. Keep the container tightly closed and in a well ventilated environment; use recommended protective equipment and wash thoroughly after handling.
- Hypersensitivity may occur, especially in persons already hypersensitive to other tetracyclines, and immediate medical attention must be sought. Persons with a known history of allergy (especially to antibiotics) and/or asthma should avoid exposure to this material. Material is irritating to mucous membranes and respiratory tract upon direct contact of higher concentrations.

[redacted]

8. ECOLOGICAL EFFECTS

Not aplicable. Product intended for human medical uses

9. FURTHER INFORMATION

- Persons handling doxycycline hyclate should be aware that other presently unknown potential hazards may exist apart from those listed herein.

The information provided in this document, which has been prepared in accordance with DIN 52 900, concerns solely bulk doxycycline hyclate and is not intended to cover situations where the doxycycline hyclate is mixed with other products. It has been prepared from available literature sources and is correct to the best of our knowledge, but any recommendations or suggestions which may be made are without guarantee, since conditions of use are outside of our control. Therefore, it is the responsibility of those persons working with, or planning to work with doxycycline hyclate to ascertain for themselves the safety aspects of such an activity.

Appendix 3: MSDS for Microcrystalline Cellulose

MATERIAL SAFETY DATA SHEET

This document has been prepared to meet the requirements of the U.S. OSHA Hazard Communication Standard, 29 CFR 1910.1200; the EEC Directive, 91/155/EEC and other regulatory requirements.

1. Company and Product Identification

[redacted]

Chemical Name : Microcrystalline Cellulose
Brand Name : [redacted]
Chemical Family : Carbohydrate
Formula : $(C_6H_{10}O_5)_x$
Synonyms : Microcrystalline Cellulose, MCC

[redacted]

2. Composition/Information on Ingredients

Ingredient Name	CAS #	EEC Symbol and Risk Phrases
Microcrystalline Cellulose	9004-34-6	Not classified as dangerous

3. Hazards Identification

Emergency Overview:

Accumulation of overhead settled dust may form explosive concentrations in air when disturbed and dispersed.

Potential Health Effects:

Minimally irritating to the eyes and non-irritating to the skin. No adverse human effects known.

4. First Aid Measures

Eyes : Flush with water for at least 15 minutes. If irritation occurs and persists, obtain medical attention.
Skin : Wash with plenty of soap and water. Get medical attention if irritation occurs and persists.
Inhalation : Remove to fresh air. If breathing difficulty or discomfort occurs and persists, obtain medical attention.
Ingestion : Drink plenty of water. Never give anything by mouth to an unconscious person. If any discomfort persists, obtain medical attention.
Notes to Medical Doctor: This compound has very low toxicity. Treatment is symptomatic and supportive only.

5. Fire Fighting Measures

Extinguishing Media : Water

Unusual Fire and Explosion Hazard : Accumulation of overhead settled dust may form explosive concentrations in air when disturbed and dispersed. The propagation of flame through air-floated dusts takes place usually following a small explosion which shakes down accumulated dust. According to NFPA 68 (Explosion Venting Guide), the Hazard Class of Dust Deflagnations for microcrystalline cellulose is St-1, the lowest hazard class.

Special Fire Fighting Procedures : For fires involving this material, do not enter any enclosed or confined fire space without wearing full protective clothing and self-contained breathing apparatus (SCBA) approved for firefighting. This is necessary to protect against the hazards of heat, products of combustion and oxygen deficiency. Do not breathe smoke, gases or vapors generated.

Hazardous Decomposition Products : None known

6. Accidental Release Measures

Maintain good housekeeping practices to minimize accumulation of settled dust, especially on overhead surfaces. Sweep up the spilled material and dispose of in accordance with the waste disposal method outlined in Section 13, "Disposal Considerations".

7. Handling and Storage

Use local exhaust or general dilution ventilation to control exposure to dust. Always use safe lifting techniques when manually moving containers, especially when shipping containers weighing more than 50 pounds (22.7 kg). To protect quality, store in a tight container in a dry place.

8. Exposure Controls/Personal Protection

Recommended Personal Protective Equipment

Respiratory : Whenever dust in the worker's breathing zone cannot be controlled with ventilation, workers should wear respirators which are approved by NIOSH/MSHA (or equivalent agency) for protection against airborne dust.

Eyes : Whenever airborne dust concentrations are high, appropriate protective eyewear, such as monogoggles, should be worn to prevent eye contact.

Gloves : Not required.

Special Clothing and Equipment : Not required.

Exposure Limits

Exposure Limit: Cellulose

	Inhalable Dust	Respirable Dust	STEL
Belgium (TWA)	10 mg/m3	–	–
France (TWA)	–	10 mg/m3	–
Switzerland (TWA)	–	6 mg/m3	–
United Kingdom (TWA)	10 mg/m3	5 mg/m3	20 mg/m3
USA (ACGIH TWA)	10 mg/m3	–	–
USA (OSHA TWA)	15 mg/m3	5 mg/m3	–

9. Physical/Chemical Properties

Appearance	: White, free flowing powder	Solubility in Water (% by Weight)	: Insoluble
Odor	: Odorless	Evaporation Rate (butyl acetate = 1)	: Not applicable
Melting Point	: Not applicable	Flash Point	: Not applicable
Boiling Point	: Not applicable	Flammable Limits (Air)	
		Upper	: Not applicable
Vapor Pressure	: Not applicable	Lower	: Not applicable
Vapor Density (Air = 1)	: Not applicable	Autoignition Temperature	
		Minimum Ignition Temp	: 420°C
pH (as is)	: Not applicable	Explosive Properties	: St-1
pH (in soln)	: 5.0-7.0 as an 11% solids dispersion	Oxidizing Properties	: Not applicable
Specific Gravity (H_2O = 1)	: Bulk density, 0.3 g/cc	Partition Coefficient (Kow)	: Not applicable
% Volatiles by Volume	: Approximately 5% water, by weight	Fat Solubility	: Not available

10. Stability and Reactivity

Stability : Stable

Hazardous Decomposition Products : None known

Conditions/Materials to Avoid (Incompatibility): None known

11. Toxicological Information

Eye Contact : Minimally irritating (rabbit). FMC Study Numbers I82-621, I82-626.

Skin Contact : Non-irritating, Primary Irritation Index (rabbit) = 0/8.0. FMC Study Number I82-625. Non-sensitizing (guinea pig). FMC Study Number I91-1184.

Skin Absorption : Dermal LD50 > 2 g/kg (rabbit). FMC Study Numbers I82-620, I82-624.

Inhalation : No mortality at maximum attainable concentration. 4 hour LC50 > 5.05 mg/l (rat). FMC Study Numbers I82-622, I82-627.

Ingestion : Oral LD50 > 5 g/kg (rat). FMC Study Number I82-623.

Acute Effects From Overexposure : No significant hazard in animal toxicity tests.

Chronic Effects From Overexposure : A 90 day animal study showed no adverse effects when administered in the diet (FMC Study Number I92-1464). This product was negative (non-mutagenic) in the Ames test (FMC Study Number I91-1189). No adverse human effects known. Microcrystalline cellulose is considered an inert dust which is not toxic to the lung when exposures are properly controlled.

Carcinogenicity: IARC: No NTP: No Other (OSHA, ACGIH): No

12. Ecological Information

Environmental Fate: Biodegradation in soil: Inherently biodegradable (FMC Study Number I92-1300).

Environmental Effects:

Rainbow Trout: 96 hr LC50 > 100%, Saturated solution. (NOEC = 100%), FMC Study Number I92-1297.

Daphnia: 48 hr LC50 > 100%, Saturated solution (NOEC = 100%), FMC Study Number I92-1298.

Algae: 96 hr EC50 > 100%, Saturated solution (NOEC = 12.5%), FMC Study Number I92-1299.

13. Disposal Considerations

No special disposal methods are suggested. It is the user's responsibility to comply with all applicable local, state, and federal laws, rules, regulations, and standards.

14. Transportation Information

U.S. DOT	: Not regulated in Title 49 of the U.S. Code of Federal Regulations as a hazardous material.
Shipping Name	: National Motor Freight Classification Item 71390, Flour Cellulose, Edible.
UN (IMO/IMDG)	: Not Applicable
Marpol Designation	: None
Canada (TDG)	: Not Applicable

15. Regulatory Information

U.S. TSCA Inventory	: Yes
U.S. SARA Title III	
Section 311/312	: None
Section 313 (40 CFR 372)	: This product does not contain any toxic chemicals subject to the reporting requirements of Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA) and 40 CFR part 372.
California Proposition 65	: This product does not contain any chemicals currently on the California list of known carcinogens and reproductive toxins.
Canada WHMIS	: Not a controlled product under the Canadian Workplace Hazardous Materials Information System (WHMIS).
EEC EINECS No.	: 232-674-9 Cellulose 231-595-7 Hydrochloric acid Note: Under the EINECS reporting guidelines, the reactants are reportable; the post-reacted natural polymer is not reportable.
EEC Symbols	: Not classified as dangerous
EEC Risk Phrases	: Not classified as dangerous
EEC Safety Advise Phrases	: Not classified as dangerous
Additional Regulatory Information	: [redacted] Microcrystalline Cellulose meets the standards set forth in the United States Pharmacopeia/National Formulary, European Pharmacopoeia, British Pharmacopoeia, The Pharmacopoeia of Japan and the Food Chemicals Codex. Microcrystalline cellulose is generally recognized as safe (GRAS) by qualified experts and is in accordance with the United States Food and Drug Administration. [redacted] maintains a Drug Master File at the U.S. Food and Drug Administration to support the safe use of [redacted] in drug products. The [redacted] products are manufactured in accordance with Current Good Manufacturing Practice and are in compliance with the Federal Food, Drug and Cosmetic Act, as Amended.

16. Other Information

NFPA Designation 704

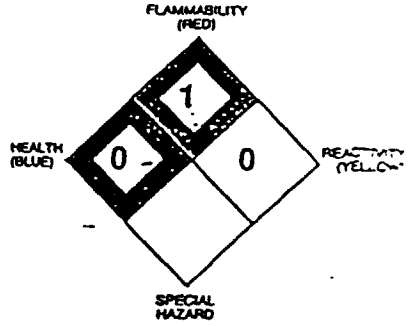
		Degree of Hazard	Degree of Hazard Code
Red	Fire:	1	4 = Extreme
Blue	Health:	0	3 = High
Yellow	Reactivity:	0	2 = Moderate
White	Special Hazard:	None	1 = Slight
			0 = Insignificant

Prepared by: [redacted]
Sections Revised: New Format

[redacted]

MATERIAL SAFETY DATA

NFPA Designation 704



DEGREE OF HAZARD
4 = EXTREME
3 = HIGH
2 = MODERATE
1 = SLIGHT
0 = INSIGNIFICANT

[redacted] Microcrystalline Cellulose

[redacted]

Identification	
Information Furnished By	[redacted]
Address	
City, State, Zip Code	
Telephone	
Product Information	
Chemical Name	Microcrystalline Cellulose
Trade Name	[redacted]
Shipping Name	National Motor Freight Classification Item 71390, Flour Cellulose, Edible
Formula	$(C_6H_{10}O_5)_x$
Chemical Family	Carbohydrate
Precautionary Information	
Hazard Classification	Nonhazardous
Signal Word and Precautionary Statement	Not a health or safety hazard
Ingredients	
Chemical Abstracts Service Registry Number and Component's Name	9004-34-6 Microcrystalline Cellulose
Physical Data	
Melting Point	Not applicable
Specific Gravity (H_2O = 1)	Bulk density, 0.3 g/cc
Boiling Point	Not applicable
Solubility in Water, % by Weight	Insoluble
Vapor Pressure	Not applicable
Percent Volatiles by Volume	Approximately 4% water by weight
Vapor Density (air = 1)	Not applicable
Evaporation Rate (butyl acetate = 1)	Not applicable
Room Temperature Appearance and State	White, free flowing powder
pH, as is	Not applicable
pH, in % solution	5.0-7.0 as a 11% solids dispersion
Odor	Odorless
Fire, Explosion and Reactivity Data	
Flash Point	Not applicable
Auto-Ignition Temperature	Not available
Flammable Limits (air)	Not applicable
Extinguishing Media	Water
Special Fire Fighting Procedures	For fires involving this material, do not enter any enclosed or confined fire space without proper protective equipment. This may include self-contained breathing apparatus to protect against the hazardous effects of normal products of combustion or oxygen deficiency.
Degree of Fire and Explosion Hazard	
Stability	Stable
Hazardous Decomposition Products	None is known
Unusual Fire and Explosion Hazards	Remote possibility of a dust explosion. If mixed with air in the proper proportions, it can be explosive (similar to flour or starch).

[redacted]

Routes of Exposure	
Inhalation	No significant hazard. No mortality in rats at maximum attainable concentration. 4 Hour LC_{50} > 5.05 – 5.49 mg/l. [redacted] Study Numbers I82-622 and I82-627 (1983).
Skin Contact	Nonirritant. Primary Irritation Index (Rabbit) = 0/8.0. [redacted] Study Number I82-625 (1982).
Eye Contact	Minimally irritating (Rabbit). [redacted] Study Numbers I82-621 and I82-626 (1982).
Skin Absorption	No significant hazard. Dermal LD_{50} (Rabbit) > 2 g/kg.
Ingestion	[redacted] Study Numbers I82-620 and I82-624 (1982). No significant hazard. Oral LD_{50} (Rat) > 5 g/kg. [redacted] Study Number I82-623 (1982).
Effects of Overexposure	No significant hazard in animal toxicity tests. No adverse human effects known.
Emergency and First Aid Recommendations	
Eyes	Flush with clean water for at least fifteen minutes. If irritation occurs and persists, obtain medical attention.
Skin	Wash with soap and water. If irritation occurs and persists, obtain medical attention.
Inhalation	Remove victim to fresh air. If breathing is difficult or if any discomfort persists, obtain medical attention.
Ingestion	Drink plenty of water. If any discomfort persists, obtain medical attention.
Special Protection Information	
Ventilation Requirements	Use only in systems, processes, and procedures in which effective ventilation has been provided.
Recommended Personal Protection Equipment	Product is not a health hazard.
Respiratory	No significant hazard. In excessive concentrations of dust wear NIOSH/MSHA approved respiratory protection.
Eyes	Appropriate eye protection, such as chemical type goggles should be worn to prevent eye contact.
Gloves	Not required
Special Clothing and Equipment	Not required
Storage and Handling	To protect quality, store in a tight container in a dry place.
Disposal, Spill or Leak Procedures	
Procedure for Release or Spill	Maintain good housekeeping practices to minimize accumulation of settled dust. Sweep up the spilled material and dispose of in accordance with the "Waste Disposal Method" given below.
Waste Disposal Method	No special disposal methods are suggested. It is the users' responsibility to comply with all applicable local, state, and federal laws, rules, regulations, and standards.
Transportation Data	Not listed in Title 49 of the U.S. Code of Federal Regulations as a hazardous material.
Additional Regulatory Information	[redacted] a purified and partially depolymerized cellulose, is listed as *Microcrystalline Cellulose* in the United States National Formulary and the Food Chemicals Codex. Microcrystalline cellulose is generally recognized as safe (GRAS) by the United States Food and Drug Administration.

Effective Date November 1, 1985

Avicel and FMC—FMC trademarks

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Appendix 4: MSDS for Magnesium Stearate

[redacted]

Material Safety Data Sheet

[redacted] provides the information contained herein in good faith but makes no representation as to its comprehensiveness or accuracy. Individuals receiving the information must exercise their independent judgment in determining its appropriateness for a particular purpose. [redacted] MAKES NO REPRESENTATIONS, OR WARRANTIES, EITHER EXPRESS OR IMPLIED, OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE WITH RESPECT TO THE INFORMATION SET FORTH HEREIN OR TO THE PRODUCT TO WHICH THE INFORMATION REFERS. ACCORDINGLY, [redacted] WILL NOT BE RESPONSIBLE FOR DAMAGES RESULTING FROM USE OF OR RELIANCE UPON THIS INFORMATION.

[redacted]

MAGNESIUM STEARATE

PRODUCT IDENTIFICATION:

Synonyms: Stearic acid, magnesium salt; octadecanoic acid, magnesium salt

Formula CAS No.: 557-04-0

Molecular Weight: 591.2 (pure)

Chemical Formula: $Mg(C_{18}H_{35}O_2)_2$

Hazardous Ingredients:
Magnesium Stearate. The exact product composition depends on purity of the tallow used.

PRECAUTIONARY MEASURES

CAUTION! MAY FORM COMBUSTIBLE DUST CONCENTRATIONS IN AIR.

Store in a tightly closed container.
Avoid dust cloud in presence of an ignition source.
Wash thoroughly after handling.

As part of good industrial and personal hygiene and safety procedure, avoid all unnecessary exposure to the chemical substance and ensure prompt removal from skin, eyes and clothing.

EMERGENCY/FIRST AID

SEE SECTION 5.

DOT Hazard Class: Not Regulated

SECTION 1 Physical Data

Appearance: Fine white to yellow-white powder.

Odor: Slight odor of fatty acid.

Solubility: Insoluble in water, ether; soluble in hot alcohol.

Boiling Point: No information found.

Melting Point: 150-170°C (302-338°F)

Specific Gravity: 1.03

Vapor Density (Air=1): Not applicable.

Vapor Pressure (mm Hg): Not applicable.

Evaporation Rate: Not applicable.

SECTION 2 Fire and Explosion Information

Fire:
Minimum dust cloud ignition temperature:
690°C (1274°F)

Explosion:
Fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard. Maximum explosion pressure: 68 lb./sq. in. at 0.1 ounces per cubic foot.

Fire Extinguishing Media:
Water spray, dry chemical, alcohol foam, or carbon dioxide. Water or foam may cause frothing.

Special Information:
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode. Pressure from the extinguishing media may cause severe dusting. Melted fatty acid can give "grease" type fire.

SECTION 3 Reactivity Data

Stability:
Stable under ordinary conditions of use and storage.

Hazardous Decomposition Products:
Burning may produce carbon monoxide and magnesium oxide.

Hazardous Polymerization:
Will not occur.

Incompatibilities:
Acids which break up the salts.

SECTION 4 Leak/Spill Disposal Information

Remove all sources of ignition. Ventilate area of leak or spill. Clean-up personnel may require protection from inhalation of dust.

Spills: Clean up spills in a manner that does not disperse dust into the air. Use non-sparking tools. Pick up spill for recovery or disposal and place in a closed container.

Disposal: Whatever cannot be saved for recovery may be burned in an approved incinerator or disposed in an approved waste facility.

Ensure compliance with local, state and federal regulations.

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SECTION 5 Health Hazard Information

A. EXPOSURE / HEALTH EFFECTS

Inhalation:
Symptoms from excessive inhalation of dust may include coughing and difficult breathing.

Ingestion:
Low level of toxicity by ingestion.

Skin Contact:
No information found. Not expected to be a hazard.

Eye Contact:
May cause mechanical irritation.

Chronic Exposure:
Grossly excessive and chronic inhalation of the dust may cause a progressive chemical pneumonitis.

Aggravation of Pre-existing Conditions:
Persons with pre-existing skin disorders, impaired respiratory function, or a history of pulmonary disease should not be exposed to dusts.

B. FIRST AID

Inhalation:
Remove to fresh air. Get medical attention for any breathing difficulty.

Ingestion:
Give several glasses of water to drink to dilute. If large amounts were swallowed, get medical advice.

Skin Exposure:
Wash exposed area with soap and water. Get medical advice if irritation develops.

Eye Exposure:
Wash thoroughly with running water. Get medical advice if irritation develops.

C. TOXICITY DATA (RTECS, 1986)

No LD50/LC50 information found relating to normal routes of occupational exposure.

SECTION 6 Occupational Control Measures

Airborne Exposure Limits:
-ACGIH Threshold Limit Value (TLV):
10 mg/m^3 of total dust.

Ventilation System:
A system of local and/or general exhaust is recommended to keep employee exposures below the Airborne Exposure Limits. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. Please refer to the ACGIH document, "Industrial Ventilation, A Manual of Recommended Practices", most recent edition, for details.

Personal Respirators: (NIOSH Approved)
If the TLV is exceeded, a dust/mist respirator may be worn up to ten times the TLV. Consult respirator supplier for details.

Skin Protection:
Gloves and lab coat, apron or coveralls.

Eye Protection:
Use chemical safety goggles. Contact lenses should not be worn when working with this material. Maintain eye wash fountain and quick-drench facilities in work area.

Avoid dust dispersal.

SECTION 7 Storage and Special Information

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Separate from incompatibilities.

.....

MAGNESIUM STEARATE NF REVISION OF:09-01-89

[redacted]

SHIP TO:

[redacted]

[redacted]

[redacted]

----------EMERGENCY ASSISTANCE----------

FOR EMERGENCY ASSISTANCE INVOLVING CHEMICALS CALL CHEMTREC (800)424-9300

----------FOR PRODUCT AND SALES INFORMATION----------

CONTACT YOUR LOCAL VAN WATERS & ROGERS BRANCH OFFICE

----------PRODUCT IDENTIFICATION----------

PRODUCT NAME: MAGNESIUM STEARATE CAS NO.: 557-04-0
COMMON NAMES/SYNONYMS:
STEARIC ACID; MAGNESIUM SALT; OCADECANOIC ACID; MAGNESIUM SALT;

FORMULA: MG (C18 H35 O2)2
HAZARD RATING (NFPA 704 CRITERIA)

HEALTH: 0
FIRE: 1
REACTIVITY: 0
SPECIAL: NONE

DATE ISSUED: 08/89
SUPERCEDES: 10/87

HAZARD RATING SCALE:
0=MINIMAL 3=SERIOUS
1=SLIGHT 4=SEVERE
2=MODERATE

----------HAZARDOUS INGREDIENTS----------

			EXPOSURE LIMITS, PPM			
			OSHA	ACGIH	OTHER	
COMPONENT	CAS NO.	%	PEL	TLV	LIMIT	HAZARD
MAGNESIUM STEARATE	557-04-0	>99	NONE	NONE	NONE	NONE

----------PHYSICAL PROPERTIES----------

BOILING POINT, DEG F: N/A
MELTING POINT, DEG F: 302-338
SPECIFIC GRAVITY (WATER=1): 1.03
APPEARANCE AND ODOR:
FINE WHITE TO YELLOW-WHITE POWDER.

VAPOR PRESSURE, MM HG/20 DEG C: N/A
VAPOR DENSITY (AIR=1): N/A
WATER SOLUBILITY, %: INSOLUBLE
EVAPORATION RATE (BUTYL ACETATE=1): N/A

----------FIRST AID MEASURES----------

IF INHALED: REMOVE TO FRESH AIR. GIVE ARTIFICIAL RESPIRATION IF NOT BREATHING. GET IMMEDIATE MEDICAL ATTENTION.

IN CASE OF EYE CONTACT: IMMEDIATELY FLUSH EYES WITH LOTS OF RUNNING WATER FOR 15 MINUTES, LIFTING THE UPPER AND LOWER EYELIDS OCCASIONALLY. GET IMMEDIATE MEDICAL ATTENTION.

IN CASE OF SKIN CONTACT: IMMEDIATELY WASH SKIN WITH LOTS OF SOAP AND WATER. REMOVE CONTAMINATED CLOTHING AND SHOES; WASH BEFORE REUSE. GET MEDICAL ATTENTION IF IRRITATION PERSISTS AFTER WASHING.

[redacted]

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IF SWALLOWED: IF CONSCIOUS, IMMEDIATELY INDUCE VOMITING BY GIVING 2 GLASSES OF WATER AND STICKING A FINGER DOWN THE THROAT. GET IMMEDIATE MEDICAL ATTENTION. DO NOT GIVE ANYTHING BY MOUTH TO AN UNCONSCIOUS OR CONVULSING PERSON.

----------HEALTH HAZARD INFORMATION----------

PRIMARY ROUTES OF EXPOSURE: INHALATION, SKIN OR EYE CONTACT.

SIGNS AND SYMPTOMS OF EXPOSURE
INHALATION: BREATHING DUST MAY IRRITATE THE NOSE AND THROAT AND CAUSE COUGHING AND CHEST DISCOMFORT.

EYE CONTACT: DUSTS MAY IRRITATE THE EYES.

SKIN CONTACT: PROLONGED OR REPEATED CONTACT WITH THE DUST MAY IRRITATE THE SKIN.

SWALLOWED: NONE CURRENTLY KNOWN.

CHRONIC EFFECTS OF EXPOSURE: NO SPECIFIC INFORMATION AVAILABLE.

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE: NONE REPORTED.

----------TOXICITY DATA----------

ORAL: NO DATA FOUND.

DERMAL: NO DATA FOUND.

INHALATION: NO DATA FOUND.

CARCINOGENICITY: THIS MATERIAL IS NOT CONSIDERED TO BE A CARCINOGEN BY THE NATIONAL TOXICOLOGY PROGRAM, THE INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, OR THE OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION

OTHER DATA: NONE.

----------PERSONAL PROTECTION----------

VENTILATION: GENERAL ROOM VENTILATION.

RESPIRATORY PROTECTION: A RESPIRATOR IS NORMALLY NOT REQUIRED IF THIS PRODUCT IS USED WITH ADEQUATE VENTILATION.

EYE PROTECTION: SAFETY GLASSES WITH SIDE SHIELDS. IT IS GENERALLY RECOGNIZED THAT CONTACT LENSES SHOULD NOT BE WORN WHEN WORKING WITH CHEMICALS BECAUSE CONTACT LENSES MAY CONTRIBUTE TO THE SEVERITY OF AN EYE INJURY.

PROTECTIVE CLOTHING: LONG-SLEEVED SHIRT, TROUSERS, SAFETY SHOES, RUBBER GLOVES, AND RUBBER APRON.

OTHER PROTECTIVE MEASURES: AN EYEWASH AND SAFETY SHOWER SHOULD BE NEARBY AND READY FOR USE.

----------FIRE AND EXPLOSION INFORMATION----------

FLASH POINT, DEG F: N/A FLAMMABLE LIMITS IN AIR, %
METHOD USED: N/A LOWER: N/A UPPER: N/A
EXTINGUISHING MEDIA: USE WATER SPRAY, DRY CHEMICAL, CO2, OR ALCOHOL FOAM.

SPECIAL FIRE FIGHTING PROCEDURES: FIRE FIGHTERS SHOULD WEAR SELF-CONTAINED BREATHING APPARATUS AND FULL PROTECTIVE CLOTHING. USE WATER SPRAY TO COOL NEARBY CONTAINERS AND STRUCTURES EXPOSED TO FIRE.

UNUSUAL FIRE AND EXPLOSION HAZARDS: AVOID DUST CLOUD IN THE PRESENCE OF AN IGNITION SOURCE.

----------HAZARDOUS REACTIVITY----------

STABILITY: STABLE POLYMERIZATION: WILL NOT OCCUR
CONDITIONS TO AVOID: HEAT, SPARKS, AND OPEN FLAMES.

MATERIALS TO AVOID: ACIDS

HAZARDOUS DECOMPOSITION PRODUCTS: MAY LIBERATE CARBON MONOXIDE, CARBON DIOXIDE AND MAGNESIUM OXIDE.

----------SPILL, LEAK, AND DISPOSAL PROCEDURES----------

ACTION TO TAKE FOR SPILLS OR LEAKS: WEAR PROTECTIVE EQUIPMENT INCLUDING RUBBER BOOTS, RUBBER GLOVES, RUBBER APRON, AND A FULL FACEPIECE OR A HALF MASK AIR-PURIFYING CARTRIDGE RESPIRATOR WITH PARTICULATE FILTERS. WEAR CHEMICAL GOGGLES IF A HALF MASK IS WORN. FOR SMALL SPILLS, SWEEP UP AND DISPOSE OF IN DOT-APPROVED WASTE CONTAINERS. FOR LARGE SPILLS, SHOVEL INTO DOT-APPROVED WASTE CONTAINERS. KEEP OUT OF SEWERS, STORM DRAINS, SURFACE WATERS, AND SOIL.
COMPLY WITH ALL APPLICABLE GOVERNMENTAL REGULATIONS ON SPILL REPORTING, AND HANDLING AND DISPOSAL OF WASTE.

DISPOSAL METHODS: DISPOSE OF CONTAMINATED PRODUCT AND MATERIALS USED IN CLEANING UP SPILLS OR LEAKS IN A MANNER APPROVED FOR THIS MATERIAL. CONSULT APPROPRIATE FEDERAL, STATE AND LOCAL REGULATORY AGENCIES TO ASCERTAIN PROPER DISPOSAL PROCEDURES.
NOTE: EMPTY CONTAINERS CAN HAVE RESIDUES, GASES AND MISTS AND ARE SUBJECT TO PROPER WASTE DISPOSAL, AS ABOVE.

----------SPECIAL PRECAUTIONS----------

TORAGE AND HANDLING PRECAUTIONS: STORE IN A DRY, WELL-VENTILATED LACE AWAY FROM INCOMPATIBLE MATERIALS. KEEP CONTAINER TIGHTLY CLOSED WHEN NOT IN USE. DO NOT USE PRESSURE TO EMPTY CONTAINER. WASH THOROUGHLY AFTER HANDLING. DO NOT GET IN EYES, ON SKIN, OR ON CLOTHING.

REPAIR AND MAINTENANCE PRECAUTIONS: NONE.

OTHER PRECAUTIONS: CONTAINERS, EVEN THOSE THAT HAVE BEEN EMPTIED, WILL RETAIN PRODUCT RESIDUE AND VAPORS. ALWAYS OBEY HAZARD WARNINGS AND HANDLE EMPTY CONTAINERS AS IF THEY WERE FULL.

----------FOR ADDITIONAL INFORMATION----------

[redacted]

----------NOTICE----------

[redacted] EXPRESSLY DISCLAIMS ALL EXPRESS
IR IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR
URPOSE, WITH RESPECT TO THE PRODUCT OR INFORMATION PROVIDED HEREIN.**

LL INFORMATION APPEARING HEREIN IS BASED UPON DATA OBTAINED FROM THE
ANUFACTURER AND/OR RECOGNIZED TECHNICAL SOURCES. WHILE THE INFORMA-
ION IS BELIEVED TO BE ACCURATE, [redacted] MAKES NO REPRESENTATIONS AS TO
TS ACCURACY OR SUFFICIENCY. CONDITIONS OF USE ARE BEYOND [redacted] CON-
ROL AND THEREFORE USERS ARE RESPONSIBLE TO VERIFY THIS DATA UNDER
HEIR OWN OPERATING CONDITIONS TO DETERMINE WHETHER THE PRODUCT IS
UITABLE FOR THEIR PARTICULAR PURPOSES AND THEY ASSUME ALL RISKS OF
HEIR USE, HANDLING, AND DISPOSAL OF THE PRODUCT, OR FROM THE PUBLICA-
ION OR USE OF, OR RELIANCE UPON, INFORMATION CONTAINED HEREIN. THIS
NFORMATION RELATES ONLY TO THE PRODUCT DESIGNATED HEREIN, AND DOES NOT
ELATE TO ITS USE IN COMBINATION WITH ANY OTHER MATERIAL OR IN ANY OTHER
ROCESS.

----------REVISION----------

8/89: CHANGED HEADING AND CONTACT INFORMATION.

**** E N D O F M S D S ****

[redacted]

Appendix 5: Certification of Compliance with 29 CFR 1900

14. References:

*1. Interim Guidance to the Pharmaceutical Industry for Environmental Assessment Compliance Requirements for the FDA. Pharmaceutical Manufacturers Association. July 1991.

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* Copies of these references have not been provided in this submission.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 9, 1975, from the *School of Pharmacy, Amer University of Beirut, Beirut, Lebanon.*

Accepted for publication April 5, 1976.

Supported in part by a grant from the University Medical Resea Fund, American University of Beirut, Beirut, Lebanon.

[x] To whom inquiries should be directed.

Oxidation Photosensitized by Tetracyclines

JOY ANNE WIEBE and DOUGLAS E. MOORE [x]

Abstract □ Irradiation with 365-nm UV light of aerated aqueous solutions of tetracycline gives rise to oxygen uptake when the pH of the solution is above 7.5. The kinetics of the reaction were followed using a polarographic oxygen electrode at a range of pH values for seven currently prescribed tetracyclines. Variation of tetracycline concentration, UV light intensity, and temperature showed the characteristics normally associated with a sensitized photo-oxygenation mechanism rather than a free-radical process. Copper(II) ions inhibited the photo-oxidation of tetracycline, apparently by complex formation. The tetracyclines were tested for photosensitizing capability with oxidizable acceptors. In aqueous solution, no photosensitizing effect could be seen, but methanol solutions of 2,5-dimethylfuran and *dl*-limonene were oxidized at considerably increased rates when small amounts of tetracyclines were present. This observation has implications for the mechanism of *in vivo* photosensitivity reactions that occur when tetracyclines are taken internally.

Keyphrases □ Tetracyclines, various—photosensitized oxidation, reaction kinetics followed using polarographic oxygen electrode, effect of pH and copper(II) ions □ Photosensitized oxidation—various tetracyclines, reaction kinetics followed using polarographic oxygen electrode, effect of pH and copper(II) ions □ Oxidation, photosensitized—various tetracyclines, reaction kinetics followed using polarographic oxygen electrode, effect of pH and copper(II) ions □ Polarography—determination, rate of oxygen uptake by solutions of various tetracyclines irradiated with UV light, effect of pH and copper(II) ions □ Antibacterial agents—various tetracyclines, photosensitized oxidation, reaction kinetics followed using polarographic oxygen electrode, effect of pH and copper(II) ions

Photosensitivity reactions have been reported for the tetracyclines after exposure of the patient to strong sunlight (1). The phenomenon of "photodynamic action" has been recognized since 1900, when it was discovered that microorganisms can be killed when exposed to light in the presence of oxygen and sensitizing dyes (2). The basic mechanism is the photosensitized oxidation of the adsorbate or substrate by molecular oxygen (3, 4). All tetracycline antibiotics have a strong absorption in the near UV region at about 365 nm, the first requirement for a sensitizer of photodynamic action.

Little information is available, however, concerning the reactions of tetracyclines following absorption of UV light. Chlortetracycline, tetracycline, and oxytetracycline lost significant antibiotic potency when irradiated with visible light in solution with riboflavin, which probably acted as a photosensitizer (5). Leeson and Weidenheimer (6) reinvestigated this system at pH 4.5 and concluded that the loss of tetracycline activity could be suppressed by the addition of ascorbic acid. Thus, an oxidative pathway was implied for degradation of tetracycline after irradiation.

This paper reports a more detailed study of the photooxidation of seven currently prescribed tetracyclines, together with some experiments that indicate that the tetracyclines can act as photosensitizers for the oxidation of suitable acceptor molecules.

EXPERIMENTAL

Samples of tetracycline[1] (I), oxytetracycline[1], doxycycline[1], metha

[1] Pfizer Laboratories.

Table I—Initial Rate of Oxygen Uptake by Irradiated Tetracycline Solutions at pH 9.0 and 30°

[Tetracycline], $M \times 10^4$	Initial Rate[a], moles/liter/min $\times 10^6$	Transmittance[b] at 373 nm, %
0.5	1.7 ± 0.2	17.8
1.0	3.5 ± 0.1	3.2
1.5	5.3 ± 0.3	<0.01
3.0	5.8 ± 0.2	<0.01
5.0	5.8 ± 0.2	<0.01
6.0	5.7 ± 0.3	<0.01
10.0	5.4 ± 0.2	<0.01

[a] Mean of at least three determinations from initial slope of P_{O_2} recorder trace. [b] Calculated for 1-cm path length and molar absorptivity of $1.5 \times 10^4\ M^{-1}\ cm^{-1}$ at pH 9.0.

cycline[1], chlortetracycline[2], demeclocycline[2], and minocycline[2] of the purest grade available commercially were used without further purification. The absorbance ratio method of McCormick *et al.* (7) showed that the purity of tetracycline, oxytetracycline, and chlortetracycline was at least 99%.

Rates of oxygen uptake by solutions irradiated by UV light were determined using the polarographic oxygen electrode apparatus described previously (8). In the procedure used, 2 ml of freshly prepared solution of I in buffer was mixed with 200 ml of air-saturated buffer solution before filling the reaction vessel. Buffers (0.05 *M*) were prepared in double-distilled water using analytical grade materials as follows: pH 4.0–4.9, acetate; pH 6.0–8.4, phosphate; pH 9.0, tromethamine; and pH 10.0–10.8, carbonate.

For experiments in which the tetracyclines were tested as photosensitizers, benzyl alcohol[3], benzaldehyde[3], *dl*-limonene[4], and 2,5-dimethylfuran[5] were purified by distilling twice under high vacuum in an all-glass apparatus at room temperature. Sodium xanthine[6], α-tocopherol[6], *dl*-tyrosine[6], and *l*-phenylalanine[6] were used as received. Limonene, dimethylfuran, and α-tocopherol are almost insoluble in aqueous systems, so experiments with these compounds were performed in methanol solution.

The rate data were recorded with the oxygen electrode operating successfully in methanol, although it was designed for operation in aqueous phases only (9). The validity of oxygen uptake data measured in methanol solution was verified by experiments with benzaldehyde for which the mechanism is known (8). Moreover, the stability of the oxygen electrode and the reproducibility of readings were greatly improved in methanol solutions; this finding may be attributed to the greater concentration of oxygen in air-saturated methanol ($2.06 \times 10^{-3}\ M$) compared to air-saturated water ($2.35 \times 10^{-4}\ M$) at 30° (10).

RESULTS

Preliminary Measurements with Tetracycline—The rate of oxygen uptake by UV-irradiated I in solution was examined over pH 4.0–10.8. No significant oxygen uptake occurred below pH 8, although there were changes in absorbance[7] at pH 4 and 5 in the 240–280-nm range, corresponding to epimerization (11). The loss of antibiotic activity of irradiated I reported (6) at pH 4.5 is thus largely due to epimerization, which occurs only between pH 2 and 6 (11).

During irradiation at pH 9.0, oxygen consumption was accompanied by color change of the solution from yellow to pink, red, or brown (depending on the extent of oxidation), with the appearance of a broad absorption centered at 530 nm. The absorbance decreased at 373 nm, which is the λ_{max} for I at pH 9.0, and disappeared completely if irradiation was continued. There was no absorption in the 400–450-nm region, indicating that neither acid degradation product (12) (anhydrotetracycline or epianhydrotetracycline) was formed during photo-oxidation. The oxygen uptake from irradiated I solutions continued (at a much reduced rate) after the lamp had been switched off, indicating that the product(s) of I photo-oxidation are labile. Analysis of these solutions with iodine immediately after irradiation produced no evidence of peroxides.

[1] Lederle Laboratories.
[2] British Drug Houses, Poole, England.
[3] Eastman Kodak, Rochester, N.Y.
[4] Fluka AG, Switzerland.
[5] Nutritional Biochemicals Corp., Cleveland, Ohio
[7] Varian Techtron model 635 UV–visible spectrophotometer.

Table II—Effect of Edetate Disodium and Copper(II) Ions on Tetracycline Photo-Oxidation at pH 9.0 and 30°[a]

[Edetate Disodium], $M \times 10^3$	$[Cu^{2+}]$, $M \times 10^3$	Initial Rate, moles of Oxygen/liter/min $\times 10^6$
0	0	3.5 ± 0.1
10	0	3.5 ± 0.2
0	1.0	2.2 ± 0.1
0	2.5	1.46 ± 0.10
0	5.0	1.17 ± 0.10
0	40	0.1 ± 0.1

[a] [Tetracycline] = $10^{-4}\ M$ in all experiments.

Correlation of the amount of oxygen consumed with the depletion of I, as indicated by the spectral change at 373 nm, established that 1 mole = 0.08 moles of oxygen reacted per mole of I. These experiments were performed at two concentrations of I; to keep secondary reactions to a minimum, irradiation was continued until no more than 15% of the dissolved oxygen was consumed.

To test for general acid or base catalysis by the buffer components, a characteristic of the epimerization reaction (7), the irradiation of I was performed at pH 9.0 in: (*a*) 0.05 *M* tromethamine buffer, (*b*) 0.05 *M* carbonate buffer, and (*c*) unbuffered solution adjusted to pH 9.0 with 1 *M* NaOH solution. After 20 min of irradiation, the pH of the unbuffered solution had fallen to 8.6 but the initial rate of oxygen uptake was the same for all three solutions, indicating that there is no participation by the buffers in the reaction nor any effect of ionic strength.

Temperature variation over 25–40° showed that the reaction rate was independent of temperature. The incident light intensity was varied as previously described (8), and the reaction rate was directly dependent. Both these observations are characteristic of a primary photochemical process rather than a free radical chain mechanism (13).

Variation of Tetracycline Concentration—Table I shows the measured initial rate of oxygen uptake as the I concentration was varied from 5×10^{-5} to $10^{-3}\ M$. Straight-line (*i.e.*, zero-order) recorder traces of oxygen pressure (P_{o_2}) *versus* time were obtained for [I] ≥ $10^{-4}\ M$. Below $10^{-4}\ M$, the traces were curved, but straight lines were obtained for plots of log P_{o_2} *versus* time. The solubility of oxygen in dilute buffer is $2.35 \times 10^{-4}\ M$ (10), so I concentration was rate limiting here and the rate can be said to be first order in I concentration. At the lower I concentrations, the rate was proportional to [I]; above $2 \times 10^{-4}\ M$, the light intensity was the limiting factor, as indicated by the percent transmittance values given in Table I. This effect also was observed previously (5, 6).

Effect of Edetate Disodium and Copper on Tetracycline Photo-Oxidation—It is well known that traces of metal ions catalyze radical chain oxidation (14) and that I complexes with many metal ions (15). The experiments summarized in Table II showed that the addition of edetate disodium to the buffer had no effect on the photo-oxidation rate, indicating that there are no accelerating effects of trace metal ions. The addition of copper(II) ions, however, led to a decrease in rate, indicating that complexed I is not susceptible to photo-oxidation. The fact that a complex was formed was indicated by a shift in the absorption maximum of I from 373 to 405 nm. The absorbance of the complex at 365 nm (the wavelength of maximum output of the UV lamp) remained sufficient to initiate photo-oxidation, provided that the complex was susceptible.

Comparative Study of Seven Tetracyclines—The initial rates of photo-oxidation for seven tetracyclines at a range of pH values are recorded in Table III. Minocycline showed little reactivity under the present conditions; methacycline also reacted more slowly than the other five tetracyclines. In general, the reaction rate was at a minimum at neutral pH and increased as the alkalinity of the solution was raised. Doxycycline showed a maximum oxidation rate at pH 9.0, while tetracycline exhibited a similar maximum at pH 10.0.

The UV spectral changes described earlier for tetracycline were observed for the other members of the group, the extent of the change in the 360–380-nm peak being directly proportional to the oxygen uptake. Chlortetracycline alone had a significant dark reaction, not involving oxygen uptake but involving a 10% reduction in absorbance of the 360-nm peak at pH 9.0 in 1 hr, the time course of the photo-oxidation reaction. Hughes and Wilson (16) showed that isochlortetracycline forms upon heating alkaline solutions of chlortetracycline while other tetracyclines are less labile in this respect.

Tetracyclines as Photosensitizers—The fact that I is the primary UV-absorbing species for its own photo-oxidation suggests that it may

Table III—Initial Rates of Photo-Oxidation of Tetracyclines at 30° and Various pH Values

Compound	Concentration, x 10^4 M	Initial Rate[a] of Oxygen Uptake (moles/liter/min x 10^4) at pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0	pH 10
Tetracycline	0.5	0	0.23	0.82	1.72	2.08	1.67
	2.0	0	0.24	1.40	5.3	6.2	4.4
Oxytetracycline	0.5	0	0.10	0.60	1.44	1.76	—
	2.0	0	0.24	1.35	3.1	4.2	—
Chlortetracycline	0.5	0	0.32	1.02	1.70	1.84	—
	2.0	0	0.89	1.6	3.3	4.2	—
Demeclocycline	0.5	0	0.22	0.8	1.33	2.22	—
	2.0	0	0.55	1.3	2.32	4.1	—
Doxycycline	0.5	0	0.66	1.4	1.68	1.12	—
	2.5	0	0.82	3.1	4.3	1.96	—
Methacycline	0.5	0	0.20	0.32	0.51	0.82	—
	2.0	0	0.32	0.73	1.20	1.32	—
Minocycline	0.5	0	<0.1	0.14	0.20	0.30	—
	2.0	0	<0.1	0.18	0.32	0.40	—

[a] Mean of three determinations from initial slope of P_{O_2} recorder trace.

be capable of sensitizing the photo-oxidation of other molecules. Various concentrations (10^{-5}–10^{-4} M) of I were tested for sensitizing capability with the following systems:

System 1—Benzyl alcohol or benzaldehyde (2×10^{-2} M) in aqueous solution at pH 4.0 and 9.0, as representative of compounds susceptible to free radical oxidation (8).

System 2—Xanthine, phenylalanine, or tyrosine (10^{-2} M) in aqueous solutions at pH 6.0 and 8.0. Both purines (17) and aromatic amino acids (18) undergo sensitized photo-oxidation in alkaline solution.

System 3—α-Tocopherol (2×10^{-2} M), a natural antioxidant that undergoes both free radical autoxidation (19) and dye-sensitized photo-oxidation (20) in methanol solution.

System 4—2,5-Dimethylfuran or *dl*-limonene in methanol solution. These agents were used (21, 22) previously as model compounds in dye-sensitized photo-oxygenations and chemical oxygenations to test for the participation of singlet molecular oxygen.

For Systems 1–3, the oxygen uptake upon irradiation was very slight and corresponded to that recorded in control experiments with I or substrate alone. Dimethylfuran and limonene, however, were oxidized significantly in the presence of all tetracyclines except minocycline (Table IV). A 10-fold change in [I] produced less than double the rate of limonene oxidation, while the doubling of doxycycline increased the rate of dimethylfuran oxidation by only 6%. Also, the absorbance of the reaction mixtures at 370 nm decreased by only about 2%, and the tetracyclines by themselves in methanol did not absorb measurable amounts of oxygen in the time course of the irradiation (1 hr). These observations establish the role of the tetracyclines as photosensitizers.

Table IV—Photo-Oxidation of Limonene and 2,5-Dimethylfuran in Methanol Solution Sensitized by Tetracyclines at 30°

Sensitizer	Concentration x 10^4 M	Substrate Concentration, M	Rate of Oxygen Uptake, moles/liter/min x 10^4
	Limonene		
None	—	0.062	0.2
Tetracycline	0.1	0.062	1.8
Tetracycline	1.0	0.062	3.2
Oxytetracycline	1.0	0.062	2.1
Doxycycline	1.0	0.062	2.4
Chlortetracycline	1.0	0.062	3.8
Demeclocycline	1.0	0.062	3.7
Methacycline	1.0	0.062	2.6
Minocycline	1.0	0.062	0.5
	2,5-Dimethylfuran		
None	—	0.038	<0.2
Tetracycline	0.5	0.038	3.4
Tetracycline	0.5	0.055	4.2
Tetracycline	0.5	0.104	7.5
Oxytetracycline	0.5	0.055	2.7
Doxycycline	0.5	0.055	2.0
Doxycycline	1.0	0.055	2.2
Chlortetracycline	0.5	0.055	3.5
Demeclocycline	0.5	0.055	3.6
Methacycline	0.5	0.055	2.8
Minocycline	0.5	0.055	1.2

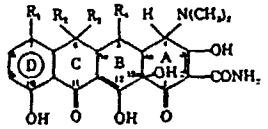
DISCUSSION

The major kinetic characteristics observed for the photo-oxidation of I in aqueous alkaline solution may be summarized as follows: (*a*) dependence on [I] only at low concentrations, (*b*) direct dependence on incident light intensity, (*c*) independence of temperature, and (*d*) use of oxygen in a 1:1 stoichiometry with I. These are the characteristics of a photosensitized oxygen addition or photo-oxygenation process (2) rather than of a free radical chain mechanism such as occurs with benzaldehyde (8).

The structures of the tetracyclines in this study are given in Table V together with the known pKa values. While it is difficult to correlate differences in structure with the differences in photo-oxidative reactivity, the rate variation with pH is clearly related to the state of ionization of the tetracyclines in mildly alkaline solution and is similar to the pH profile observed for the photo-oxidation of several aromatic amino acids (18). The pKa values are attributed (23) respectively to the tricarbonyl system of ring A (pK_1), the dimethylammonium function of ring A (pK_2), and the phenolic β-diketone system in the C-10–11–12 region (pK_3). From the pKa values, I is said to exist as a zwitterion at neutral pH and increasingly as the monoanion as the pH is raised above 8. On this basis, the monoanion would appear to be the reactive species.

The photo-oxidation reaction is accompanied by a loss of the absorption peak at about 370 nm, which is due to the chromophore grouping in the BCD ring (7, 24) containing the ionizable system corresponding to pK_3. The dissociation of this group is essentially unaffected by the 7-chloro substitution, but it is not known whether the different substituents

Table V—Structures and pKa Values of Tetracyclines



Name	R_1	R_2	R_3	R_4	pK_1	pK_2	pK_3	Reference
Tetracycline	H	CH_3	OH	H	3.30	7.68	9.69	23
Chlortetracycline	Cl	CH_3	OH	H	3.30	7.44	9.27	23
Oxytetracycline	H	CH_3	OH	OH	3.27	7.32	9.11	23
Doxycycline	H	CH_3	H	OH	3.4	7.7	9.7	30
Demeclocycline	Cl	H	OH	H	3.30	7.16	9.25	31
Methacycline	H	—CH_2—		OH	—	—	—	—
Minocycline	$N(CH_3)_2$	H	H	H	—	—	—	—

in methacycline and minocycline cause a significant alteration in the pK value and, therefore, the concentration of the monoanion. There is no marked difference in the absorption peak at 370 nm for all tetracyclines studied here.

The addition of copper(II) ions shifts the 370-nm absorption to longer wavelength and renders the complex stable to photo-oxidation at pH 9.0. This tends to implicate the C-10–11–12 grouping as the binding site for metal ions. Mitscher *et al.* (24) concluded, from optical rotatory dispersion studies, that chelation occurred first in the BCD ring and then in the A ring as the pH was raised above 10. Williamson and Everett (25) recently suggested, on the basis of NMR studies in dimethyl sulfoxide, that the tricarbonylmethane function of ring A is the chelating group, but the solvent difference may give rise to a different binding behavior.

The decreased rate of photo-oxidation above pH 10.0 for I and pH 9.0 for doxycycline are not easily attributable to the decrease in the concentration of the monoanion in solution; if the monoanion is the only oxidizable species of I, the maximum rate should occur at pH 8–9, where its concentration would be at a maximum on the basis of the pK values.

The demonstration that the tetracyclines are capable of acting as photosensitizers for the oxygenation of suitable acceptor molecules has relevance to the photosensitivity reactions observed following the ingestion of I and its analogs. Dimethylfuran and limonene are acceptors (A) of energy transferred from $^1O_2$, the excited singlet molecular oxygen (21). This implies that excited I is capable of interacting with $^3O_2$ (ground-state triplet oxygen) to give singlet oxygen according to Scheme I

$$(I) \xrightarrow{h\nu} (I)^*$$

$$(I)^* + {}^3O_2({}^3\textstyle\sum_g^-) \rightarrow (I) + {}^1O_2({}^1\Delta_g)$$

$$^1O_2 + A \rightarrow AO_2$$

Scheme I

This phenomenon has been principally studied using dyes that absorb in the visible region, such as methylene blue or rose bengal, since the energy difference between the triplet oxygen ground state and the singlet excited state is only 92 kJ (22 kcal), which corresponds to a wavelength of 1270 nm (21). It also usually requires that the dye pass to the excited triplet state, which has a longer lifetime in which to effect the energy transfer.

Foote (26) stated that if the oxidation proceeds purely through singlet oxygen as an intermediate, it should produce the same rate for all acceptors at constant sensitizer and oxygen concentrations. Thus, the similarity in rates observed with limonene and dimethylfuran is in agreement with this concept. No photosensitizing capability of I could be demonstrated in aqueous solution, so it is clear that the acceptor or substrate molecule and solvent medium have to be specifically matched to the sensitizer for greatest efficiency. Young *et al.* (27) suggested that the lifetime of singlet oxygen in photosensitized oxidations increases as the polarity decreases. This aspect could not be tested, since the amino acids and purines are insufficiently soluble in methanol and limonene and dimethylfuran are insoluble in water.

Reports of *in vivo* photosensitivity have implicated demeclocycline more frequently than the other members of the group (28, 29). The results in Table IV indicate some differences in photosensitizing efficiency, with demeclocycline, chlortetracycline, and tetracycline being the most efficient. The relative inactivity of minocycline is due to a shift of the λ_{max} in methanol solution to 345 nm, so that its molar absorptivity at 365 nm is approximately half that of the other tetracyclines. The investigation is continuing with systems that can be related more closely to the skin.

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Temperature and concentration dependent partitioning of three tetracyclines between phosphate buffers and octanol

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Colaizzi & Klink (1969) analysed in detail the pH-dependence of the apparent partition coefficients (P) of several tetracyclines at 25° and one initial concentration of the drugs in the aqueous phase in an attempt to interpret qualitatively the differences in biological activities of this homologous series of compounds. The partition coefficients of the zwitterionic form have been calculated from these data, and incorporated in a review by Leo, Hansch & Elkins (1971). We have found that the values of P may vary significantly with temperature, and also with drug concentration in the region studied by Colaizzi & Klink (1969). Such a dependence may be of importance in quantitative correlations of 'lipophilicity' with drug transport properties, protein binding and biological activity.

In our work the drugs were dissolved in octanol-saturated phosphate buffer of defined ionic strength, pH 7·5 at 25°; any minor deviation in pH was corrected at this stage. The solutions were shaken vigorously with buffer-saturated octanol in a shaking bath at the required temperature for 2 h. Samples from the aqueous phase were then diluted suitably, and analysed spectrophotometrically at 274 nm (tetracycline and doxycycline hydrochlorides) and 282 nm (methacycline hydrochloride), using phosphate-saturated buffer as reference. The values of P were calculated as the ratio of the concentration in the octanol phase divided by the concentration in the aqueous phase, the former value obtained from mass balance.

Table 1. *Apparent partition coefficients of methacycline hydrochloride (I) between phosphate buffer (pH 7·5 at 25°, ionic strength 0·09 M) and octanol and of doxycycline hydrochloride (II) between phosphate buffer (pH 7·5 at 25°, ionic strength 0·045 M) and octanol at various initial concentrations of the drugs in the buffer, and at several temperatures.*

Initial concn of drug (mg %)	Temperature (°C)	Apparent partition coefficients I	II
20	25	0·45	0·67
	29	0·42	—
	31	—	0·59
	33	0·37	—
	37	0·33	0·57
30	25	0·51	0·70
	29	0·44	—
	31	—	0·59
	33	0·38	—
	37	0·34	0·58
40	25	0·50	0·75
	29	0·47	—
	31	—	0·65
	33	0·46	—
	37	0·35	0·63

* Correspondence.

Table 1 shows a decrease in P of methacycline and doxycycline hydrochlorides with increasing temperature and decreasing drug concentration. In Table 2, the difference between P values at 25° and 37° is indicated for the three tetracyclines, the conditions used being otherwise similar to those of Colaizzi & Klink (1969).

As shown by Colaizzi & Klink (1969), a small decrease from pH 7·5 results in higher values of P. The pH of our buffer decreases by about 0·03 units as a result of the temperature increase of 12°. It is evident that the trends in Tables 1 and 2 cannot be explained on the basis of this change of pH. In fact, Cooke (1976) concluded that the temperature dependence of the second ionization constant of tetracyclines, which determines the overall contribution of the most lipophilic ionic species of these compounds, i.e., the zwitterion concentration (Leeson, Krueger & Nash, 1963; Colaizzi & Klink, 1969), is unlikely to account fully for the observed dependence of the apparent partition coefficients.

The temperature dependence of the intrinsic (i.e. single species) octanol/water partition coefficients has been reported to be small, of the order of 0·01 log P

Table 2. *Apparent partition coefficients of three tetracyclines between phosphate buffer (pH 7·5 at 25°) and octanol at 25° and 37°.*

Drug	Initial concn in buffer (mg %)	Ionic strength of buffer (M)	Apparent partition coefficient 25°	37°
Tetracycline HCl	52	0·18	0·038	0·026
Methacycline HCl	20	0·09	0·45	0·33
Doxycycline HCl	20	0·09	0·60	0·51

For comparison Colaizzi & Klink (1969) found the following P values at pH 7·5, 25°, ionic strength of the phosphate buffer 0·1M, drug concentration $5{\cdot}203 \times 10^{-4}$ M (approx. equivalent to 25 mg %): tetracycline hydrochloride 0·036, methacycline hydrochloride 0·43, and doxycycline hyclate 0·60. Full details with results can be found in Cooke (1976).

(intrinsic) per °C (Leo & others, 1971). Care must be exercised, however, in applying this statement. Firstly, for compounds with small absolute values of log P, the difference between the *in vitro* values (usually determined at room temperature) and the *in vivo* magnitude at 37° can lead to a large error for the purpose of quantitative *in vitro—in vivo* correlations Secondly, many compounds of medicinal interest have pKa values with temperature dependences (Ballard, 1974) that can have a profound effect on the proportion of their most lipophilic forms present, and thus on partitioning results. Such effects have been reviewed by Ballard (1974). Recently, temperature dependence of partition coefficients has been reported by Kaufman, Semo & Koski (1975), Davis, Elson & others (1976) and Dearden (1976).

Finally, in view of the complex ionization scheme (Leeson & others, 1963) and partitioning behaviour (Colaizzi & Klink, 1969) of tetracyclines, it would be rather tedious to extract thermodynamic values of the relevant physicochemical parameters for the purpose of correlating these quantitatively with biological activity of these compounds. We suggest that, instead, the apparent partition coefficients of tetracycline and its analogues should be measured under conditions closely resembling the biological activity studied.

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The effects of solubility and method of drying on the drug content of various size fractions of tablet granules

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Variations of drug content of different size fractions of granules have been reported (Lachmann & Sylwestrowicz, 1964; Cox, Ambaum & Wijnand, 1968; Travers, 1974; Selkirk, 1976). These variations have been considered to be due to solvent migration during drying, and abrasion of the granule surface during subsequent handling. We find that this is an insufficient explanation of some results we have obtained.

To examine the effects of the solubility of the minor component and the method of drying, a series of 1 kg batches of granules were made using lactose B.P. (Whey Products Ltd.) as diluent and either sulphanilamide (ICI) or sulphacetamide sodium (Ward Blenkinsop) as drug. The damp granules were divided into two sub-batches and then dried using either a fluid-bed drier or a standard laboratory oven with a fan. A 100 g sample of each sub-batch of dried granules was sieved, and one coarse and some fine sieve fractions were assayed for drug content.

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Preparation of granules. The granules were made by one of two methods. In method 1, the component powders were screened together. For the 0·02 and 1% levels of sulphacetamide sodium, and the 0·02% level of sulphanilamide, the required weight of drug for a 1 kg batch was dissolved in 120 ml of the binder solution, 5% w/v aqueous polyvinylpyrrolidone (K29-32Gaf). For sulphanilamide 1 and 2%, drug equivalent to 0·02% was dissolved in the binder solution and the remaining sulphanilamide was mixed with the lactose in a Morton Z blade mixer for 2 min before binder was added. The binder (plus drug) was added in two 60 ml portions with 1 min massing between additions; there was a further 5 min massing following the addition of the second portion of the binder. The damp mass was forced through a 1·0 mm screen using a Jackson-Crockatt granulator and divided into 4 portions by quartering. Two opposite quarters were combined for fluid bed drying at 50° for 25 min and one quarter was placed in an enamel tray (20 cm × 30 cm) for oven drying at 50° for 90 min. The dried granules, which had