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Shyamal K. Majumdar, Ph.D.
Editor
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# Contents

**Journal Information** 2

**Articles**

**BIOLOGY: Second Pass Metabolism and Transport of Acetylsalicylic Acid and Acetaminophen in the Vascularly Perfused Rat Intestine** 3
Daryl M. Simmons

**BIOLOGY: Impacts of Prey Preference by *Daphnia pulex* on Algal Productivity** 9
William L. Keough, Nancy McCreary Waters and Carla M. Delucchi

**BIOLOGY: Habitat Use and Differential Foraging Behavior in the Downy Woodpecker *(Picoides pubescens)*** 15
Fred J. Brenner, Brant E. Fisher and Daniel Laferrierre

**BIOLOGY: Dual Therapy in the Treatment of Gonorrhea and its Effect on the Prevalence of *Chlamydia trachomatis* Infection** 18
David H. Much and Johnson W. Martin

**CHEMISTRY: An Investigation Into the Bioisosterism of Vinyl and Cyclopropyl Groups in Local Anesthetics** 21
James A. Beres, Joy Umstead Daggett, Scott L. Dax, Janet Mueller, Beth A. Dombroski, Gregory G. Graboski, Timothy J. Schram, Bradley H. Diehl, William R. True, Colleen M. O'Neill, Jean Hemminger Conroy and Marjorie R. Kubiak

**GEOGRAPHY: Directional Profiles and Rose Diagrams to Complement Centrographic Cartography** 29
Mark Monmonier

**PSYCHOLOGY: A Concept-Coordinated Escape Reflex in the Mongolian Gerbil** 35
Timothy L. Cleary

**RESEARCH NOTE: Does the Color Pattern of Two Species of Turtles Imitate Duckweed?** 39
David A. Ross and Jeffrey E. Lovich

**COMMENTARY: A Training Course for Lead Abatement Workers and Supervisors** 43
John H. Lange

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(Continued to inside back cover)

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# SECOND PASS METABOLISM AND TRANSPORT OF ACETYLSALICYLIC ACID AND ACETAMINOPHEN IN THE VASCULARLY PERFUSED RAT INTESTINE[1]

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## ABSTRACT

**Using the rat, an in-situ, vascular intestinal perfusion model, was developed. The rat mesenteric vasculature was perfused with 1 mL of a saturated aqueous methylene blue solution to confirm the surgical integrity of the preparation and identify the circulatory path from mesenteric artery to hepatic portal vein during development of the method. The test compounds, acetylsalicylic acid (ASA) and acetaminophen (APAP) were perfused in 1 mL volumes of 2.5 $\mu$moles. Blood samples were collected from the hepatic portal vein and the inferior vena cava. The intestinal lumen was rinsed with 1 mL of physiological saline (0.85%) at the end of the infusion to remove the lumenal contents. HPLC assays were conducted on the blood samples, lumenal rinses and scrapings of the intestinal mucosa.**

**ASA and salicylic acid (SA) were detected in the lumenal perfusate, mucosa, and hepatic portal blood samples. Only SA was found in the systemic blood. ASA concentrations were higher than SA in the mucosa. Methylene blue was detected in the hepatic portal and systemic bloods and found in trace amounts in the intestinal mucosa and lumenal rinses, confirming the anatomical pathway.**

**APAP and traces of acetaminophen sulfate (APAP•$SO_4$) were detected in the mucosa and lumenal perfusate. APAP was found in the hepatic portal blood but neither APAP nor APAP•$SO_4$ was detected in the systemic blood. The results verified a second intestinal metabolism of compounds in the blood and showed that compounds in the mesenteric circulation can be transported to the intestinal lumen.**
**[J PA Acad Sci 66(1):3-8, 1992]**

## INTRODUCTION

Absorption of an orally administered compound involves transport from the intestinal lumen to the mesenteric circulation. The compound is then transported in the blood via the hepatic portal vein to the liver before reaching the vena cava and achieving systemic circulation (Rowland *et al.* 1972). The molecule is subjected to possible biotransformation first by the lumenal contents, and then by several epithelial events in traversing the intestinal wall (Porteous *et al.* 1984). Brush border enzymes are associated with the microvilli of the absorptive cells and, endocytotic enzymes are active between the terminal web and basal membrane of the enterocyte (Figures 1a and 1b; Roth *et al.* 1985, Murer *et al.* 1976). Transport of a compound or its metabolite is indicative of access to a metabolic pathway. Intraarterial perfusion of an organ with a compound mimics the condition experienced by a molecule during circulation. Analysis of the organ's venous blood permits data collection revealing the metabolic disposition of the compound by the organ. This is the classic approach used in organ perfusions (Xu *et al.* 1989, Pang *et al.* 1985, Meijer *et al.* 1981, Katza and O'Brien 1979, Sakai *et al.* 1979). Compounds in the systemic circulation have been shown to undergo metabolism during a second pass through an organ's circulation, most notably the liver and the intestine (Pang *et al.* 1985). However, the authors neglected to mention methods for verifying the identification of the vasculature in development of the model. The above methods involve such procedures as ligature of the arterial blood which violates the blood supply (Katz and O'Briend 1979, Sakai *et al.* 1979). Other investigators introduced oxygenated media, or surgical alteration of anatomical obstacles to the experiment (DeVries *et al.* 1989, Pang *et al.* 1986). Those procedures necessarily altered the natural conditon to achieve extended perfusion times of up to several hours.

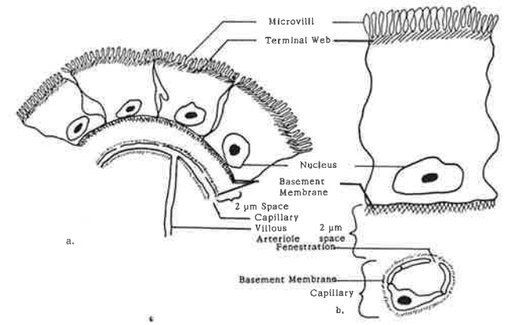


FIGURE 1. Diagrammatic representation of intestinal villi absorption cells; (a) enterocytes of a villous tip and underlying vasculature; (b) enlarged schematic of a single enterocyte with an accompanying capillary.

[1]Received for publication 17 October 1991; accepted 30 January 1992.

However, such methods may be inducing metabolic effects by excessive stress on the organs.

A method is proposed here for identification of the organ and its vasculature that does not involve radioactive markers. This procedure is a qualitative, reproducible, and comparatively uncomplicated method for quick determination of whether the intestine does metabolize a compound already absorbed into the systemic circulation.

## MATERIALS AND METHODS

### *Surgical*

Albino male Charles River, Sprague Dawley rats, ranging in weight from 380 g to 440 g, were fasted 18 hrs. prior to surgery but given water ad libitum. The animals were anesthetized with sodium pentobarbital at 50 mg/kg, i.p., and taped to a 37°C slide warmer (Precision Scientific Inc., 3737 W. Cortland St., Chicago, IL). Cannulations were made through a midline abdominal incision. Polyethylene tubing (0.50 mm, i.d., 0.80 mm, o.d.; Dural Plastics and Engineering, Dural, New South Wales, 2158, Australia), fitted with the 5 mm tip of a 25 gauge needle, was used to cannulate the superior mesenteric artery by insertion of the needle tip into the vessel (Figure 2). The placement was secured by application of one drop of instant adhesive (Super Glue Corp., Hollis, New York). The hepatic portal vein was cannulated caudal to its junction with the gastrosplenic vein. The inferior vena cava was cannulated midway between the hepatic and the renal veins.

The intestine was cannulated 1 cm below the pyloris with polyethylene tubing of the same size and manufacture as the arterial cannula. A 4.0 silk suture ligation secured the placement and prevented gastric emptying into the intestine. A second intestinal cannulation, using teflon tubing (4 mm O.D., 3 mm I.D., Waters, Milford, MA) was similarly secured at a position 30 cm distal to the duodenal cannula. Physiological saline (0.85%), containing 100 I.U./mL of sodium heparin was administered in a 1 mL volume to each animal via the arterial cannula.

The intestinal mass was wrapped in plastic film (Saran Wrap, Dow Chemical Company, Inc., P.O. Box 68511, Indianapolis, IN) to prevent drying and replaced in the opened abdomen. The intestine was perfused with 2.5 $\mu$moles of drug solution in a 1 mL volume via the arterial cannula for 2 minutes. Portal and systemic blood samples were collected in 200 $\mu$L volumes at 60 second intervals for 5 min. After a 5 minute period, the lumen was perfused with 1 mL of physiological saline. Following collection of the intestinal fluid, the animal was sacrificed with a pentobarbital overdose. The lumenally perfused intestinal segment was removed and longitudinally sectioned to expose the mucosa. The mucosae were scraped with a soft rubber spatula and the scrapings were analyzed by HPLC.

### STANDARD PREPARATION

Reference standards were prepared in triplicate for ASA, SA, and APAP. Serial dilutions in rat blood were used with

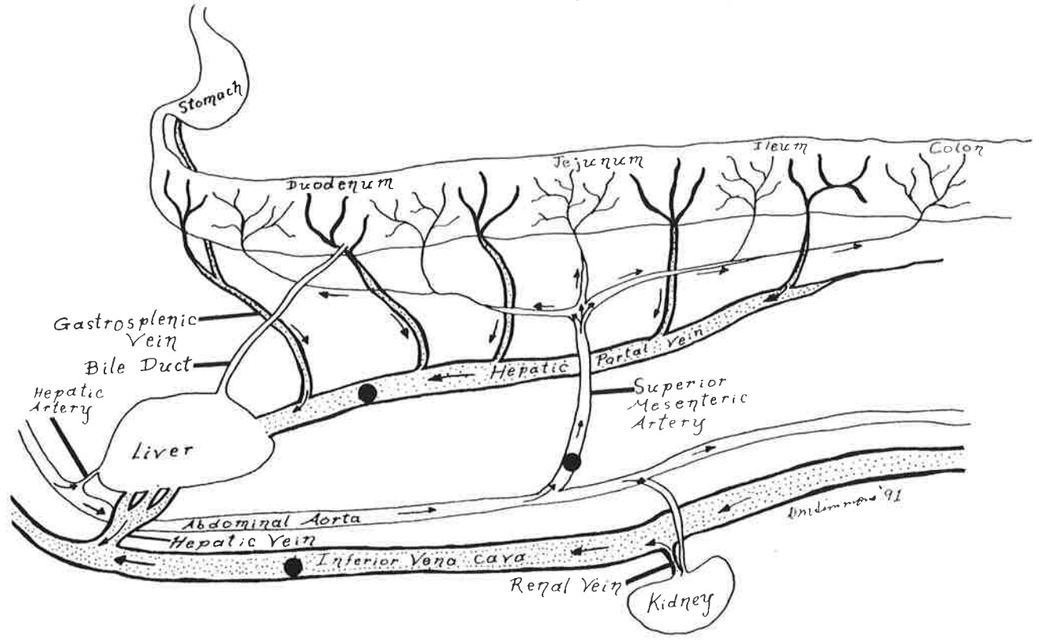


FIGURE 2. A diagram of the intestine and its vasculature. Blood flow is represented by arrows and placement of the three vascular cannulations is identified by •.



concentrations of 50, 30, 10, 0.5 and 0.05 $\mu$moles/mL to generate standard curves. The APAP•$SO_4$ reference also contained APAP and was used for retention time identification only. Similar standards were prepared in water for assays of lumenal perfusate.

### SAMPLE PREPARATION AND ANALYSIS

The HPLC conditions for ASA, SA, and APAP and APAP•$SO_4$ are outlined in Table 1. Samples of blood, lumenal perfusate and mucosal scrapings were treated and prepared the same way. For ASA and SA determinations, 200 $\mu$L of sample was added to 400 $\mu$L of acetonitrile containing 30 $\mu$g/mL of phenol as the internal standard. The preparation was vortex mixed, centrifuged at 2800 g for 20 minutes and the supernatant assayed by HPLC. For analysis of APAP and APAP•$SO_4$, 100 $\mu$L of a 0.1 mg/mL aqueous solution of tetrabutyl ammonium phosphate was added to 200 $\mu$L of sample.

The preparation was vortex mixed, and washed with three 1 mL volumes of chloroform. The supernatant was separated by centrifugation at 2800 g for 30 minutes and assayed by HPLC.

## RESULTS

The model was tested with ASA and APAP since both compounds are well documented and the metabolic activities of each is understood (Needs and Brooks 1985, Bogentoft *et al.* 1983, Levy 1979, Rowland *et al.* 1972, Rowland and Riegelman 1968, Harris and Riegelman 1967, Boyd and Bereczky 1966, Gwilt *et al.* 1963). Both ASA and APAP have low molecular weights (180 and 151 respectively), and similar simple structures, but different metabolic profiles (acetylesterase substrate versus sulfatase and glucuronidase substrate). These properties provide a basis for investigation of biotransformation mechanisms.

Aspirin eluted after phenol (internal standard) with baseline resolution and before its SA metabolite with respective retention times of 4.7, 5.3, and 7.6 minutes (Figure 3). APAP and its sulfated conjugate eluted in 5.3 and 7.6 minutes respectively (Figure 4). Biological interferences were eliminated from the chromatography in the preparative steps. This is verified by the clean baseline of the blanks. Chromatograms of extracts from mucosal scrapings are shown since these samples had the most interferences. A glucuronoide conjugate of APAP was unavailable for reference, therefore, no results for glucuronidation of the drug are reported.

Linearity of the standard curves is shown in Figure 5. Standard derivations from the mean (n = 3) are represented by vertical bars. The correlation coefficients of ASA, SA, and APAP were 0.998, 0.997, and 0.990 respectively. Quantitation of APAP•$SO_4$ was not possible, due to the presence of APAP in the reagent, and no reference curve is shown, but the retention time was used for identification of the conjugate.

Aspirin solutions in pH 6.9 phosphate buffer (0.1 M) were stable at 37°C for at least 2 hours. Acetaminophen preparations in saline at pH 6.9 were also stable at 37°C for at least 2 hours. These solutions were suitable for experimental use.

TABLE 1. HPLC Conditions for Drug Assays of Acetylsalicylic Acid (ASA), Salicylic Acid (SA), Acetaminophen (APAP) and Acetaminophen Sulfate (APAP•$SO_4$).

| | ASA and SA | APAP and APAP•$SO_4$ |
|---|---|---|
| Mobile Phase: | water-acetonitrile-85% phosphoric acid (800:200:5) | water-methanol-tetraburyl ammonium phosphate (900:100:0.08 g) |
| Flow: | 2 mL/min | 1.5 mL/min |
| Internal Standard: | Phenol | none |
| UV Detection: | 284 nM | 254 nm |
| Injection: | 50 $\mu$L (WISP, Model 712, Waters, Milford, MA) | |
| Guard Column: | RP-18 Newguard, 15 x 3.2 mm, 7 (Brownlee Labs, Inc., 9 Bridgewell Place, Clifton, NJ) | |
| Analytical Column: | Whatman 10-ODS3, 25 cm (Whatman Chemical Separations, Inc., 9 Bridgewell Place, Clifton, NJ) | |
| Integration: | Peak heights, 745 Data Module (Waters, Milford, MA) | |

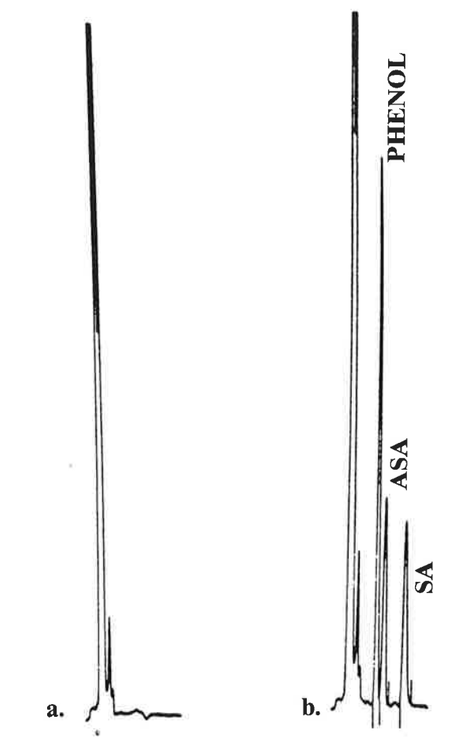


FIGURE 3. Typical HPLC chromatogram of muscosal scrapings; (a) intestine from an unmedicated rat; (b) ASA perfused intestine.

## SURGICAL PREPARATION

Identification of the mesenteric and hepatic vasculatures, and validation of the surgical model were confirmed with the saturated methylene blue solution. Injection of the dye turned the intestine blue and visibly altered the color of the portal vein sequentially. The liver then turned blue. Blood collected from the hepatic portal vein and the vena cava, was heparinized (100 IU/mL), and centrifuged to separate the plasma. The color change was not measured but the plasma of each sample was visibly blue as compared to plasma samples taken prior to administration of the dye. Traces of methylene blue were detected in the intestinal mucosa and lumenal perfusate. The dye was not used in the drug injected animals since the anatomical pathway was identified.

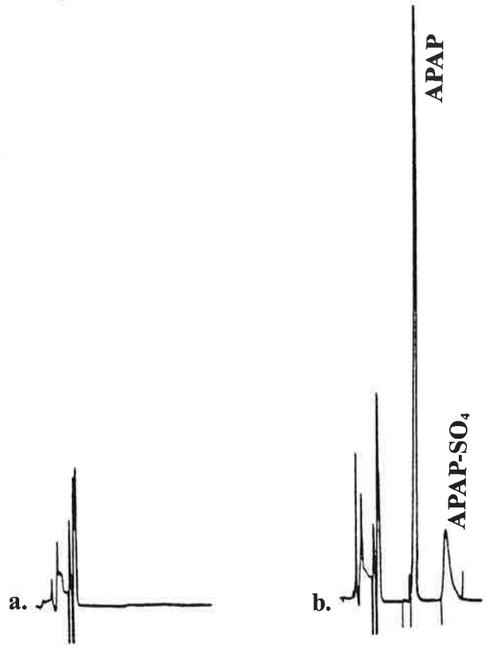


FIGURE 4. Typical HPLC chromatogram of mucosal scrapings; (a) intestine from an unmedicated rat; (b) APAP perfused intestine.

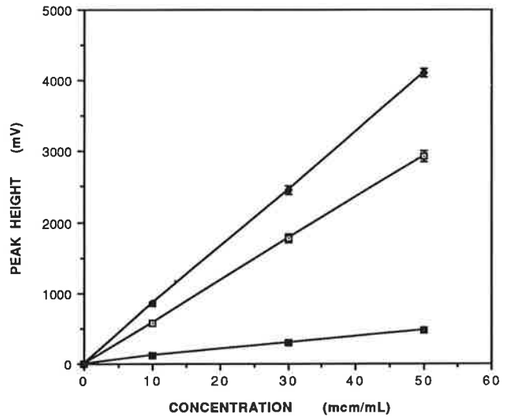


FIGURE 5. Mean standard curves for reference solutions of ASA, SA, and APAP.

The hepatic portal venous blood contained both ASA and SA (Figure 6). The minor amounts of SA in the systemic blood were subtracted from assayed values in the portal blood to obtain the corrected amounts. Only SA was detected in the systemic samples and only after 3 minutes. Absence of ASA in the systemic blood is indicative of the clearance and/or metabolism after total body circulation.

The presence of ASA in the lumenal perfusate and intestinal mucosa confirmed transport of the compound from the mesenteric vasculature to the intestinal lumen (Figure 7). The site of metabolism remains unconfirmed by these experiments.

The rat intestine is populated with a high amount of microflora (Friend and Chang 1984). The bacteria may be responsible for significant lumenal metabolism of ASA and/or APAP.

APAP•$SO_4$ was undetected in the blood samples. Portal blood levels of APAP confirmed integrity of the surgical preparation (Figure 8). Systemic levels of APAP were not detected. Acetaminophen was found in the lumenal perfusate and the mucosa, as were equal trace amounts of the sulfate conjugate (Figure 9). Sulfation, as a minor metabolic pathway in the rat, has been demonstrated in intestinal preparations. Pang (1985) demonstrated sulfation of APAP in the perfused ileum with a plasma recovery of only 0.16% of the APAP dose. In this experiment, APAP•$SO_4$ may have been beyond detectable limits in the portal blood or not readily absorbed. Sulfation might have been the result of bacterial metabolism in the lumen since APAP•$SO_4$ was undetected in the portal blood. Pang (1985) did find the sulfated conjugate in the lumenal contents of lumenally perfused rat intestine with higher amounts of APAP•$SO_4$ in the ileum than the jejunum.

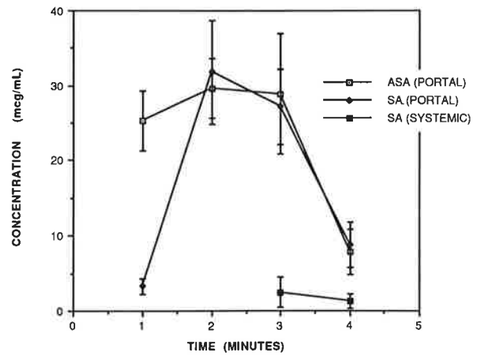


FIGURE 6. Mean blood levels of ASA and SA in the hepatic portal and systemic blood samples (n = 6).



## DISCUSSION

The presence of ASA and APAP in the lumenal perfusate and intestinal mucosa confirmed drug transport from the mesenteric vasculature to the lumen, which is the first step of Second Pass Metabolism. The presence of SA and APAP•$SO_4$ in the mucosa and lumenal perfusate confirmed metabolism but not the mechanism. Since orally administered drugs are subjected to intestinal metabolism during absorption, ASA and APAP would be subject to metabolism during reabsorption from the lumen. ASA and APAP in the hepatic portal blood would next be metabolized by the liver, completing the second step of Second Pass Metabolism.

Intestinal transport of these drugs was shown to occur in two directions; absorptive from the lumen and excretory, into the lumen.

The model does not provide data for kinetic derminations but does provide distinct advantages over in-vitro procedures in the literature. It can be set up quickly with fewer resources, minimizing the exposure of the organ to foreign fluids, and very nearly emulating the intact natural system. It also confirms that cellular transport can occur in two directions. Methylene blue can be used to verify the surgical preparation during method development without the caution required when using radiolabeled markers.

The model could be expanded for evaluation of drug metabolism by allowing longer perfusion time. Subtraction of the systemic drug levels from the hepatic portal concentrations would give corrected values for assayed amounts in the portal blood. This would yield data on intestinal clearance rates, for kinetic determinations.

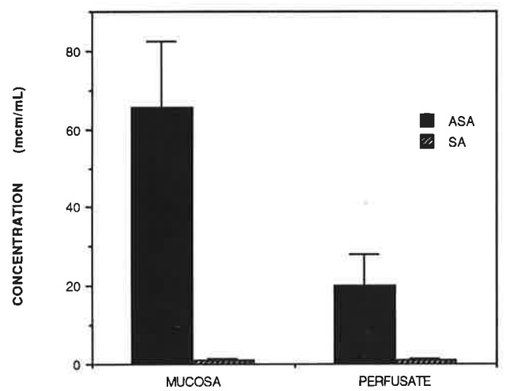


FIGURE 7. Mean acetylsalicylic acid and salicylic acid concentrations in the intestinal mucosa and lumenal perfusate (n = 6).

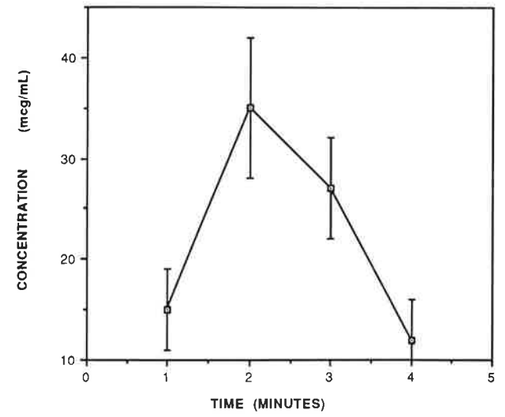


FIGURE 8. Mean hepatic portal blood levels of APAP (n = 6).

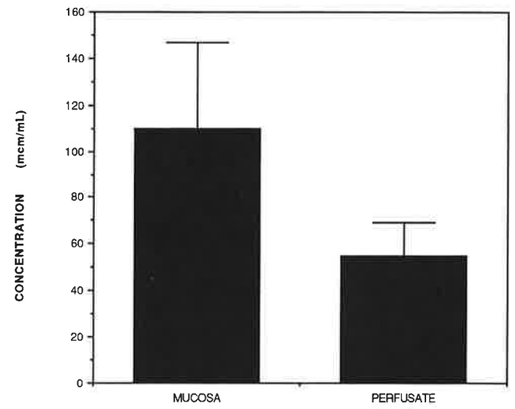


FIGURE 9. Mean acetaminophen concentrations in the rat intestinal mucosa and lumenal perfusate (n = 6).

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## LITERATURE CITED

Bogentoft, C., G. Dahl and A. Magnusson. 1983. Urinary pH and plasma levels of salicylate after administration of different buffered acetylsalicylic acid formulations. *Biopharm. Drug Disp.* 4:137-143.

Boyd, E.M. and G.M. Bereczky. 1966. Liver necrosis from paracetamol. *Brit. J. Pharmacol.* 26:606-614.

DeVries, M.H., G.A. Hofman, A.S.J. Koster and J. Noordhock. 1989. Systemic intestinal metabolism of 1-naphthol; a study in the isolated vascularly perfused rat small intestine. *Drug Met. Disp.* 17:573-578.

Friend, D.R. and G.W. Chang. 1984. A colon-specific drug-delivery sysfem based on drug glycosides and the glycosidases of colonic bacteria. 1984. *J. Med. Chem.* 27:261-266.

Gwilt, J.R., A. Robertson and E.W. McChesney. 1963. Determination of blood and other tissue concentrations of paracetamol in dog and man. *J. Pharm. Pharmacol.* 15:440-444.

Harris, P.A. and S. Riegelman. 1967. Acetylsalicylic acid hydrolysis in human blood and plasma I; Methodology and In Vitro Studies. *J. Pharm. Sci.* 56:713-716.

Katz, M. and R. O'Brien. 1979. Vitamin $B_{12}$ absorption studied by vascular perfusion of rat intestine. *J. Lab Clin. Med.* 94:817-825.

Levy, G. 1979. Pharmacokinetics of salicylate in man. *Drug Met. Rev.* 4:3-19.

Meijer, D.K.F., K. Keulemans and G.J. Mulder. 1981. Isolated perfused rat liver technique. *Meth. Enzym.* 77:81-94.

Murer, H., E. Ammann, J. Biber and U. Hopfer. 1976. The surface membrane of the small intestinal epithelial cell. I. Localization of adenyl cyclase. *Biochem. Biophys. Acta.* 433:509-519.

Needs, C.J. and P.M. Brooks. 1985. Clinical pharmacokinetics of the salicylates. *Clin. Pharmaco.* 10:164-177.

Pang, K.S., V. Yuen, S. Fayz, J.M. Tekoppele and G.J. Mulder. 1985. Absorption and metabolism of acetaminophen by the in-situ perfused rat small intestine. *Drug Met. Disp.* 14:102-111.

Porteous, J.W., J.D. Hutchinson and V. Undreill. 1984. The lumenally and vascularly perfused small intestine as an experimental system for the study of translocation and metabolism. *Proceed. Nutri. Soc.* 43:141-160.

Roth, J., M.J. Lentz and E.G. Berger. 1985. Immunocytochemical demonstration of ecto-galactosyl transferase in absorptive intestinal cells. *J. Cell. Biol.* 100:118-125.

Rowland, M., S. Riegelman, P.A. Harris and S.D. Sholkoff. 1972. Absorption kinetics of aspirin in man following oral administration of an aqueous solution. *J. Pharm. Sci.* 61:379-385.

Rowland, M. and S. Riegelman. 1968. Pharmacokinetics of acetylsalicylic acid and salicylic acid after intravenous administration in man. *J. Pharm. Sci.* 57:1313-1320.

Sakai, K., M. Akima, Y. Hinohara, M. Sasaki and R. Niki. 1979. Vascularly perfused rat small intestine: a research model for drug absorption. *Japan. J. Pharmacol.* 30:231-241.

Xu, X., H. Hirayama and K.S. Pang. 1989. First-pass metabolism of salicylamide; studies in the once-through vascularly perfused rat intestine-liver preparation. *Drug Met. and Disp.* 17:556-563.

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# IMPACTS OF PREY PREFERENCE BY *DAPHNIA PULEX* ON ALGAL PRODUCTIVITY[1]

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## ABSTRACT

**Laboratory experiments estimated the impact of different *Daphnia pulex* densities on chlorophyll *a* and phaeopigment concentrations, and primary productivity. Chlorophyll *a* declined with increasing *Daphnia* density, while phaeopigment concentrations increased linearly with increasing zooplankton density. These results are consistent with theoretical predictions regarding the importance of predator-prey interactions in aquatic food webs. Additional studies employed Carbon-14 labelling techniques to determine feeding preferences of *Daphnia* on cultures of various combinations of *Chlamydomonas* and *Chlorella*. In these studies a negative relationship occurred between grazing pressure and primary productivity, whereas food web theory predicts a unimodal relationship. Labelling experiments showed a lack of preferential feeding in *Daphnia* for either phytoplankton species. The implications of these results to theories of optimal foraging and cascading food web interactions are examined.**
**[J PA Acad Sci 66(1):9-14, 1992]**

## INTRODUCTION

Studies of how aquatic food webs are organized can be classified by the ecological approach (McQueen *et al.* 1986, 1989). One theory maintains that nutrient supply primarily controls the size, structure, and diversity of primary producers and subsequent trophic levels of consumers. As a result, limited nutrient supply affects the phytoplankton community through competition, thereby dictating composition of the zooplankton community, which in turn affects planktivore and, subsequently, piscivore composition. Since control of the aquatic food web is exerted at the base of the trophic structure, this might be termed a "bottom-up" approach.

In apparent contrast, the second theory maintains that predators significantly impact the size, structure, and diversity of their prey community, and that these feeding patterns extend down through the trophic web of consumers which ultimately affect primary producers and nutrient use and recycling. Therefore, in an aquatic ecosystem large fish dictate small fish abundance, biomass and production through predation. Since most small fish are generally planktivores, they will feed preferentially on zooplankton, thereby affecting that community composition. In turn, since zooplankton graze upon phytoplankton, they will exert considerable control over primary producers and impact nutrient recycling. Throughout the food web, selections by predators are primarily related to size of prey. Since control of the system is exerted at the top of the trophic structure, this approach is commonly referred to as a "top-down" approach to aquatic ecosystems.

Cascading trophic interactions (Carpenter *et al.* 1985) is a top-down approach which attempts to relate predator biomass to prey biomass and productivity. Cascading trophic interactions (referred to hereafter as CASCADE) predict an inverse linear relationship between predator biomass and prey biomass. Specifically, for herbivorous zooplankton/phytoplankton interactions, an increase in zooplankton biomass should cause phytoplankton biomass to decline. However, grazing by zooplankton on phytoplankton impacts rates of nutrient regeneration. When grazing is especially heavy, nutrient recycling is impaired, and overall primary production falls. Likewise, when grazing is especially low, rates of nutrient recycling are no longer stimulated and primary production is low. Therefore, CASCADE predicts that primary production should be highest at an intermediate biomass of herbivore.

In contrast to CASCADE, a bottom-up approach would predict phytoplankton community biomass is directly related to nutrient availability. Competition for limiting nutrients controls the phytoplankton community, so as nutrient supply increases, phytoplankton biomass increases. An increase in phytoplankton biomass means less competition among herbivores for food, and a resultant increase in zooplankton biomass. Patterns of primary production should be essentially similar. Although other measures are available, phytoplankton biomass is commonly measured by estimates of chlorophyll *a* concentration,

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while primary production is commonly assessed by carbon-14 labelling to determine the rate of carbon assimilation (Wetzel and Likens 1991).

CASCADE further considers how trophic levels not immediately adjacent to one another in the trophic web can impact relationships between production and biomass. For example, phosphorus supply has long been considered a good indicator of lakewide productivity and, therefore, lake trophic state. However, Schindler (1978) indicated that phosphorus supply could explain only 48% of the variation in primary production among a large data set of the world's lakes. Additionally, lakes with similar phosphorus supply rates can differ by nearly a thousandfold in primary productivity (Carpenter *et al.* 1985). Likewise, even though phosphorus loading explains 79-95% of the variance in chlorophyll *a* concentration (Dillon and Rigler 1974, Oglesby 1977, Schindler 1978, Carpenter *et al.* 1985), according to some authors, chlorophyll *a* concentration is a poor predictor of primary production (Brylinsky and Mann 1973, Oglesby 1977, Carpenter *et al.* 1985). According to CASCADE, this unexplained variation could be attributable to feeding practices of higher-level predators altering community structure of prey, effectively tumbling down through the trophic web. For instance, Brooks and Dodson (1965) demonstrated that diversity, density, and size structure of zooplankton communities can be altered through grazing by planktivorous fish.

This regulation of trophic level biomass and composition in freshwater pelagic ecosystems has received considerable attention in the recent literature (McQueen *et al.* 1989). Along with CASCADE, the biomanipulation concept (Shapiro *et al.* 1982, Shapiro 1980) also focuses on the impact of predators on community structure. Both of these top-down models predict that any change in piscivore abundance will be transferred through the food web, thereby causing indicators such as chlorophyll and primary production to deviate from predictions based solely on nutrient supply, which is essentially a bottom-up approach. In addition, productivity at all trophic levels and energy flow through the food web should be highest where intensities of predation (grazing) are intermediate (Carpenter *et al.* 1985).

Although maximum primary production may be limited by nutrient availability, herbivores may prevent this peak from being achieved. If there is differential selectivity for a given group or species of phytoplankton, composition of the community may shift to reflect that preference. It is commonly assumed that many predators forage for food sources which provide the greatest relative input of energy per unit cost of foraging. Optimal foraging theory would be classifed as a top-down approach to predator-prey interactions, and may contribute to or alter the outcome of food web interactions predicted by CASCADE. If a predator is foraging in this fashion, it will, when given a choice among prey, choose a prey with greater nutrient value until that prey type is no longer available, then the predator will switch to the next preferred prey (Schoener 1971).

Optimal foraging predicts that, given a choice, a zooplankton species should preferentially feed on one algal species over another. As the abundance of the preferred algal species declines due to increased grazing, its contribution to the diet of the grazer should exceed its contribution to the phytoplankton community. However, if proportionate composition of diet reflects the proportionate composition of available phytoplankton, then no preference is being expressed by the grazing zooplankton. One index of grazing by zooplankton is concentration of phaeopigments, which are generated by the breakdown of chlorophyll *a* pigments associated with passage through the acidic gut of zooplankton (Carpenter *et al.* 1986, Carpenter and Bergquist, 1985).

From their inception, optimal foraging studies have engendered considerable controversy (Schoener 1971), especially since a limited number of experiments have been performed or have produced strongly supporting results (Pyke 1984, Pyke *et al.* 1977). As a result, Pyke (1984) developed criteria for evaluating studies of optimal foraging theory, and determined that more experimental studies are necessary before the theory can be rigorously evaluated.

To date, the majority of studies testing predictions of CASCADE have been large scale experiments, either whole lake manipulations, or mesocosm studies of *in situ* natural assemblages of organisms. The objective of this study was to examine components of both optimal foraging theory and CASCADE predictions on a smaller scale, specifically using phytoplankton and a single zooplankton species, in an effort to rigorously test predictions of both optimal foraging and CASCADE. *Daphnia pulex* was permitted to graze upon the algae *Chlamydomonas* and *Chlorella*, to determine if phytoplankton biomass (measured by chlorophyll *a*) and primary production (measured by $H^{14}CO_3^-$ fixation) varied as predicted by cascading trophic level theory. In theory, increased *Daphnia* density should result in a proportional decrease in chlorophyll *a*, along with a unimodal response in $H^{14}CO_3^-$primary production (Carpenter *et al.* 1985). Optimal foraging experiments were then employed to determine if *Daphnia* demonstrated a preference for either *Chlamydomonas* or *Chlorella*. This choice was particularly interesting in light of some preliminary work indicating higher amino acid content in *Chlamydomonas* compared to *Chlorella* (Mineo and Silcox, pers. comm.), thereby creating a nutritional differential which might favor *Chlamydomonas*.

## MATERIALS AND METHODS

*Biomass and Productivity Estimates:*

Initial trials (n = 9) of *Daphnia pulex* employed six different densities of animals: 0, 10, 15, 30, 45, 60, or 90 zooplankton in 250 ml of filtered lake water held in a 500 ml straight sided glass jar. Each jar received as food source a 50 ml aliquot of 7-10 day old *Chlamydomonas* culture grown in Algal Grow media (Carolina Biological Supply Company). Jars were held in an incubator at 15°C and constant light through the experiment. Replicate 50 ml aliquots of *Chlamydomonas* were collected onto 0.45 $\mu$m membrane filters. These filters were extracted in acetone and absorption at 750, 665, and 663 nm was determined using a UV/VIS spectrophotometer. From these measurements, both chlorophyll *a* and phaeopigment initial concentrations were determined (Wetzel and Likens, 1991 p. 157).

After 6 hours, *Daphnia* were removed by filtering the jar contents through a 62 $\mu$m Nitex mesh screen. The resulting filtrate was subsequently collected onto 0.45 $\mu$m membrane filters, which were analyzed for chlorophyll *a* and phaeopigments using the same acetone extraction employed previously.

To measure $^{14}C$ uptake and estimate productivity, trials were repeated using the same densities of *Daphnia* and *Chlamydomonas* in filtered lake water. The 50 ml aliquot of *Chlamydomonas* was employed, using algae cultured under constant light in 10 gallons aged tap water with Peterson's plant food. Two additional treatments were included in these uptake experiments. In one treatment, 1 ml of saturated 3-(3,4-Dichlorophenyl)-1, 1-Dimethylurea (DCMU) solution was added in the absence of *Daphnia* to determine $^{14}C$ uptake by algal non-photosynthetic processes. In the second additional treatment, *Daphnia* were included at all densities, but without *Chlamydomonas*. This treatment enabled us to account for $^{14}C$ uptake directly by *Daphnia*.

Three separate trials were run using $^{14}C$ uptake methods: 0.5 $\mu$Ci (n = 6), 1 $\mu$Ci (n = 6), or 3 $\mu$Ci (n = 2) of $NaH^{14}CO_3$ were added to the experimental jars, depending on the trial. Each jar was stirred and immediately sub-sampled to determine initial counts per minute (CPM) with a Beckman scintillation counter. The jars were then incubated as indicated previously. *Daphnia* were subsequently removed by filtering through a 62 $\mu$m mesh Nitex screen, and were then transferred to a 0.45 $\mu$m membrane filter. Likewise, filtrate from each jar was collected onto a 0.45 $\mu$m membrane filter. Both filters, algae and *Daphnia*, were washed with 0.01 M HCl acid solution to remove surface adhered $H^{14}CO_3$. Filters were placed in scintillation vials, to which 0.5 ml of 0.5 M HCl was added. Filters were then dried at 65°C overnight and CPM subsequently determined as indicated previously. CPM, corrected for DCMU, were then used to calculate productivity as mg carbon fixed/cm³/hr.

*Optimal Foraging Estimates*

Thirty *Daphnia* in 500 ml jars holding 250 ml of filtered lake water were used for all foraging experiments (n = 9). Treatments consisted of mixtures of *Chlamydomonas* to *Chlorella* at 0%:100%, 25%:75%, 50%:50%, 75%:25%, and 100%:0%, respectively. Replicate 50 ml aliquots of the combined phytoplankton were analyzed to determine initial chlorophyll *a* and phaeopigments using the filtration and extraction procedures employed earlier. Jars were incubated at 15°C under constant light for 6 hours. At that time, *Daphnia* were collected onto a 62 $\mu$m Nitex mesh screen. They were then transferred in distilled water to a 0.45 $\mu$m membrane filter. The remaining contents in each jar were passed through a 0.45 $\mu$m membrane filter. Both of these filters were subsequently analyzed for chlorophyll *a* and phaeopigment.

Foraging trials were then repeated in two different $^{14}C$ labelling experiments (n = 4 for each). In the first experiment, *Chlamydomonas* was labelled by first incubating with $NaH^{14}CO_3$, and then combining the labelled *Chlamydomonas* with unlabeled *Chlorella* in the mixture ratios employed earlier. Initial estimates of CPM were made by measuring 3 replicate 1 ml aliquots of labelled *Chlamydomonas* as indicated previously in a scintillation counter. In the second experiment, *Chlorella* received the radioactive label, was combined with unlabelled *Chlamydomonas*, and initial estimates of CPM for *Chlorella* radioactivity were made. In each experiment, after the 6 hour incubation under constant light at 15°C, *Daphnia* were collected onto a 62 $\mu$m Nitex mesh filter, transferred to a 0.45 $\mu$m membrane filter, and CPM determined as indicated previously. CPM from within *Daphnia* were expressed as a percentage of CPM taken up relative to initial CPM radioactivity to estimate percent uptake. Analysis of variance (ANOVA) was used to compare differences in percent uptake between the two algal species and among the different treatment mixtures. Significant models were further analyzed using Scheffe's Multiple Comparison of Means Test. All data analyses employed the use of an IBM PS-2 equipped with PC-SAS.

## RESULTS

*Biomass and Productivity*

Initial chlorophyll *a* concentrations (n = 9) averaged 335.67 ± 9.89 (SD) $\mu$g/1. In all populations, chlorophyll *a* concentration declined with increasing *Daphnia* densities, (Figure 1) and chlorophyll *a* was virtually undetectable at densities of 45 *Daphnia*/250 ml and higher, indicating a substantial decline in algal biomass with increased grazing. Conversely, phaeopigment concentrations rose with increasing *Daphnia* density, comprising virtually all of the pigment found in filtrate at densities of 45 *Daphnia* or greater. These results suggested that 30 *Daphnia*/250 ml would exert sufficient grazing pressure for subsequent productivity experiments. Additionally, chlorophyll *a* was used to estimate grazing pressure as follows:

$$\text{Grazing Pressure} = \frac{\text{chlorophyll } a}{\textit{Daphnia} \text{ abundance} * 0.300\text{L } H_2O}$$

Since *Daphnia* uptake of $H^{14}CO_3^-$ in phytoplankton-free controls was less than 0.3% of algal uptake, the impact of direct uptake of radioactive tracer by *Daphnia* could be ignored. Likewise, DCMU treatments indicated a minor

amount of non-photosynthetic uptake of $H^{14}CO_3^-$. Nonetheless, all productivity data were adjusted for DCMU uptake. When primary productivity was plotted as a function of grazing pressure (Figure 2), linear regression demonstrated a significant, negative relationship ($R = 0.4583$, $p < 0.0001$). However, there was a significant lack of fit ($r^2 = 0.21$), indicating a substantial amount, nearly 80%, of variation unexplained by the regression. Neither a polynomial model nor a natural log transformation improved the goodness of fit of the regression.

*Optimal Foraging*

Total initial chlorophyll *a* concentrations were approximately 75% lower in foraging trials compared to biomass trials. In particular, the two species of algae exhibited a 2-3 fold difference in initial chlorophyll levels, which was also reflected in mixtures (0% *Chlamydomonas*: 100% *Chlorella* = 39.8 ± 2.6 μg/l; 100% *Chlamydomonas*: 0% *Chlorella* = 101.6 ± 8.9 μg/l). As a result, total initial pigment levels increased with increasing *Chlamydomonas* proportional composition.

Results of these foraging trials using pigment concentrations as a response showed virtually no chlorophyll *a* in the filtrate (Figure 3). Concomitantly, phaeopigments in filtrate increased with a greater proportion of *Chlamydomonas* as food source (Figure 3), indicating substantial conversion of chlorophyll *a* to phaeopigments due to grazing. Further, a maximum of about 33 μg/l phaeopigments could be attributable solely to grazing on *Chlorella*, as evidenced in the 100% *Chlorella* treatment; any increase in phaeopigment must be due to grazing on *Chlamydomonas*, which far exceeds grazing on *Chlorella*.

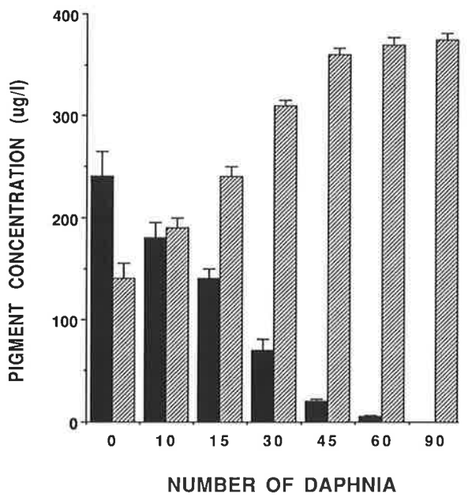


FIGURE 1. Pigment concentration as a function of increasing *Daphnia* abundance. Solid bars indicate chlorophyll *a*; hatched bars indicate phaeopigments. Each bar represents the mean of n = 9, ± 1 standard deviations.

In labelling foraging trials, however, *Chlamydomonas* concentration did not significantly affect carbon uptake within *Daphnia* ($F = 1.61$, $p = 0.2393$), even though a general pattern of increased uptake with increased *Chlamydomonas* existed (Figure 4). In experiments using labelled *Chlorella*, $^{14}C$ uptake, and by inference grazing, increased significantly with increasing *Chlorella* concentration ($F = 14.02$, $p = 0.0003$). Scheffe's comparison of means test indicated that uptake of labelled *Chlorella* in the 75%:25% *Chlamydomonas: Chlorella* was significantly lower than in the remaining labelled *Chlorella* treatment concentrations (Figure 4). Regardless, *Daphnia* ingestion of *Chlorella* was an order of magnitude lower than was ingestion of *Chlamydomonas* at comparable concentrations (Figure 4).

## DISCUSSION

The decline in chlorophyll *a*, and therefore *Chlamydomonas* biomass, with increasing *Daphnia* abundance as well as the corresponding increase in phaeopigment was consistent with results from previous laboratory and field studies (McQueen *et al.* 1989; Bergquist *et al.* 1985; Bergquist and Carpenter, 1986; Lynch and Shapiro, 1981; Kerfoot *et al.* 1988). Consistent also with the aforementioned

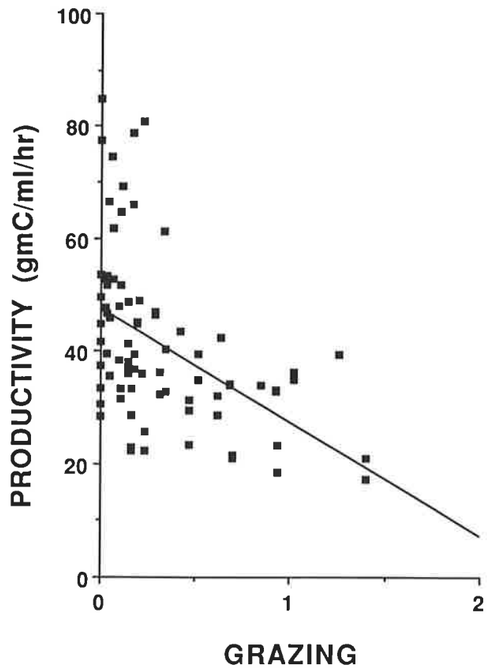


FIGURE 2. Primary productivity as a function of grazing pressure for all productivity trials using 0.5, 1, and 3 μCi. Equation for best fit linear regression is y = 47.427 - 20.021x.



studies was the inverse relationship between chlorophyll *a* and phaeopigment concentrations. Top-down models predict that as large herbivores increase, biomass of small flagellates should decline and approach zero in the water column (Carpenter and Kitchell 1984; Cooper 1973).

The same models predict a unimodal relationship between grazing pressure and primary productivity. The present study reports a significant negative relationship. This relationship could be generated by the limited range of grazing pressure incorporated in the study, effectively limiting the responses to one side of the expected unimodal curve. However, the lack of fit of the negative relationship indicates that variables in addition to grazing pressure may be impacting primary production. One possible explanation may be the limited nutrient regeneration experienced by algae in short-term, microcosm experiments.

However, a unimodal change in primary productivity as a function of intermediate densities of the grazer *Daphnia* is related to community level changes in phytoplankton. In particular, grazer resistant phytoplankton, those of large cell size or with specific grazer-deterring adaptations, should dominate the phytoplankton community, due to an accompanying shift in the taxa present in the water column. This response was not observed in our study; once standing crop of a single phytoplankton species was depleted, no other phytoplankton species were present to capitalize on the increased availability of nutrients as a result of grazing. This is part of the inherent limitations of microcosm approaches. Although more rigidly controlled for environmental variations, microcosms are limited in the degree of complexity of species interactions. Had we employed a more realistic phytoplankton community assemblage we may have been able to document a shift in species composition. However, we would not have been able to determine which species exhibited changes in chlorophyll *a*, phaeopigments, or primary productivity. Our more artificial design allowed substantially more control over the simple system such that we could actually document the precise changes in the single species *Chlamydomonas*.

The results of biomass foraging experiments suggest that *Daphnia* exhibited a preference for *Chlamydomonas* but when $^{14}C$ labelled algae was used, *Daphnia* did not demonstrate any significant feeding preferences for either *Chlamydomonas* or *Chlorella*. These labelling experiments showed that percent uptake of *Chlamydomonas* did not increase with increasing proportion of *Chlamydomonas* concentration. Paradoxically, when *Chlorella* was labelled, *Daphnia* showed substantial uptake, indicating increased grazing with increasing *Chlorella* in mixture. This difference can be attributable to the inordinately low uptake of labelled *Chlorella* when very little (only 25% of mixture) was made available to *Daphnia*. However, the overall difference in magnitude of uptake suggests that *Daphnia* do not actively select for *Chlorella* and are probably feeding more on *Chlamydomonas*.

One possible explanation for the lack of preference for *Chlorella* may be related to encounter rates (Porter, 1976; Schoener, 1971). *Daphnia* probably encounter motile *Chlamydomonas* more often then non-motile *Chlorella* which may sink to the bottom of the microcosm. Additionally,

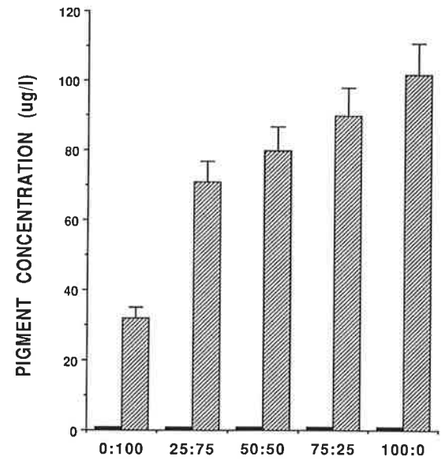


FIGURE 3. Pigment concentration found in filtrate in relation to ratio of % *Chlamydomonas:* % *Chlorella* initially added. Solid bars indicate chlorophyll *a*; hatched bars indicate phaeopigments. Each bar represents a mean of n = 9, ± 1 standard deviations.

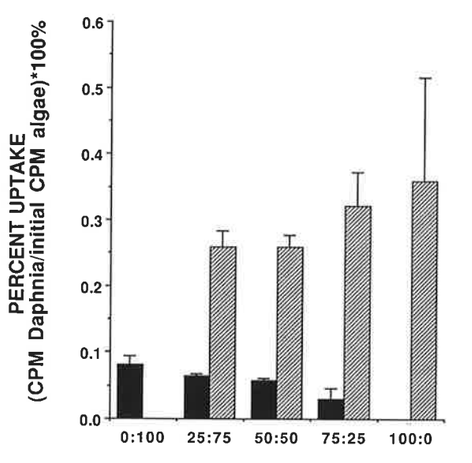


FIGURE 4. Percent uptake into *Daphnia* of labeled algae in relation to % *Chlamydomonas*: % *Chlorella*. Solid bars indicate uptake of *Chlorella*; hatched bars indicate uptake of *Chlamydomonas*. Each bar represents a mean of n = 4 ± 1 standard deviations.

some preliminary data (Mineo and Silcox, pers. comm.) indicate that *Chlamydomonas* exhibits substantially higher protein content than *Chlorella*, which may explain the difference in apparent preferences. These differences in relative nutritive content represent an avenue for further study of zooplankton preferences for phytoplankton.

In conclusion, this study supports predictions of CASCADE regarding how changes in zooplankton biomass impact phytoplankton biomass. However, predictions relating to productivity are less well supported, and require further investigation. *Daphnia pulex* did not indicate preference for *Chlamydomonas* over *Chlorella* as a prey type, but does ingest more *Chlamydomonas* probably due to encounter rates. This component of foraging requires additional study to elucidate the mechanism by which choices by grazers are made. Further laboratory manipulations should include different predator and grazer densities which approach more natural phytoplankton assemblages. Field enclosure studies, such as those reported by Bergquist *et al.* (1985), Bergquist and Carpenter (1986), and Kerfoot *et al.* (1988) compared natural systems but they lacked the degree of control and precision that is capable in a laboratory microcosm. Further optimal foraging studies should be examined particularly at the grazer/phytoplankton level, since prey selection by grazers seems to have a significant impact on phytoplankton community structure.

## ACKNOWLEDGEMENTS

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## LITERATURE CITED

Bergquist, A.M. and S.R. Carpenter. 1986. Limnetic herbivory: Effects on phytoplankton populations and primary production. Ecology 67:1351-1360.

Bergquist, A.M., S.R. Carpenter and J.C. Latino. 1985. Shifts in phytoplankton size structure and community composition during grazing by contrasting zooplankton assemblages. Limnol. Oceanogr. 30:1037-1045.

Brooks, J.L. and S.I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150:28-35.

Brylinsky, M. and K.H. Mann. 1973. An analysis of factors governing productivity in lakes and reservoirs. Limnol. Oceanogr. 18:1-14.

Carpenter, S.R. and A.M. Bergquist. 1985. Experimental tests of grazing indicators based on chlorophyll *a* degradation products. Arc. Hydrobiol. 102:303-317.

Carpenter, S.R., M.M. Elser and J.J. Elser. 1986. Chlorophyll production, degradation, and sedimentation: implications for paleolimnology. Limnol. Oceanogr. 31:112-124.

Carpenter, S.R. and J.F. Kitchell. 1984. Plankton community structure and limnetic primary production. Am. Nat. 124:159-172.

Carpenter, S.R., J.F. Kitchell and J.R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience 35:634-639.

Cooper, D.C. 1973. Enhancement of net primary productivity by herbivore grazing in aquatic laboratory microcosms. Limnol. Oceanogr. 18:31-37.

Dillon, P.J. and F.H. Rigler. 1974. The phosphorus-chlorophyll relationship in lakes. Limnol. Oceanogr. 20:767-773.

Kerfoot, W.C., C. Levitan, W.R. DeMott. 1988. *Daphnia*-phytoplankton interactions: density-dependent shifts in resource quality. Ecology 69:1806-1825.

Lynch, M. and J. Shapiro. 1981. Predation, enrichment, and phytoplankton community structure. Limnol. Oceanogr. 26:86-102.

McQueen, D.J., J.R. Post, E.L. Mills. 1986. Trophic relationships in freshwater pelagic ecosystems. Can J. Fish. Aquat. Sci. 43:1571-1581.

McQueen, D.J., M.R.S. Johannes, J.R. Post, T.J. Stewart and D.R.S. Lean. 1989. Bottom-up top-down impacts on freshwater pelagic community structure. Ecol. Monogr. 59:289-309.

Oglesby, R.T. 1977. Phytoplankton summer standing crop and annual productivity as functions of phosphorus loading and various physical factors. J. Fish. Res. Board Can. 34:2255-2270.

Porter, K.G. 1976. Enhancement of algal growth and productivity by grazing zooplankton. Science 192:1332-1334.

Pyke, G.H. 1984. Optimal foraging theory: a critical review. Annu. Rev. Ecol. Syst. 15:523-575.

Pyke, G.H., H.R. Pulliam and E.L. Charnov. 1977. Optimal foraging: a selective review of theory and tests. Quart. Rev. Biol. 52:137-154.

Schindler, D.W. 1978. Factors regulating phytoplankton production and standing crop in the world's lakes. Limnol. Oceanogr. 23:478-486.

Schoener, T.W. 1971. Theory of feeding strategies. Annu. Rev. Ecol. Syst. 2:369-404.

Shapiro, J. 1980. The importance of trophic level interactions to the abundance and species composition of algae in lakes. Pages 105-115 *in*: J. Barica and L. Mur, editors. Hypertrophic Systems. Dr. W. Junk Publishers, The Hague, The Netherlands.

Shapiro, J., B. Forsberg, V. Lamarra, G. Lindmark, M. Lynch, E. Smeltzer and G. Zoto. 1982. Experiments and experiences in biomanipulation—studies of biological ways to reduce algal abundance and eliminate blue-green. EPA-600/3-82-096. Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR.

Wetzel, R.G. and G.E. Likens. 1991. Limnological Analyses. W.B. Saunders Company, Philadelphia, PA, 391 pp.

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# HABITAT USE AND DIFFERENTIAL FORAGING BEHAVIOR IN THE DOWNY WOODPECKER (*PICOIDES PUBESCENS*)[1]

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## ABSTRACT

**Habitat selection and foraging behavior of downy woodpeckers (*Picoides pubescens*) were studied on three woodlots in western Pennsylvania and central Ohio. Downy woodpeckers preferred ecotones over forested areas on all three study sites. Of the 350 individual observations, 61.7% of the birds occurred in ecotones. Male and female downy woodpeckers exhibited different foraging behavior patterns. Males foraged primarily on the smaller branches while females spent the majority of their time foraging on the main trunks and larger branches. Sexual divergence in feeding behavior allows for a greater exploitation of available food resources while reducing competition between male and female woodpeckers.**
**[J PA Acad Sci 66(1):15-17, 1992]**

## INTRODUCTION

The downy woodpecker (*Piciodes pubescens*) occurs in woodlots throughout North America. Bent (1939) suggested that downy woodpeckers prefer open woodlands but the species has been reported foraging in a range of habitats from pole stage timber to mature forests (Conner 1980). The downy woodpecker is primarily insectivorous (Beal 1911), obtaining food primarily by drilling into bark and by gleaning the bark surfaces (Jackson 1970). The remaining portion of their diet consists of fruits, seeds and nuts.

The downy woodpecker has been reported to forage most frequently on lower branches 2.5 cm or less in diameter (Williams 1975, Conner 1981). There are seasonal differences in habitat selection by the downy woodpeckers in that during the breeding season the species apparently prefers woodlands with a mean basal area of 11.3 $m^2$/km whereas during the post breeding and winter seasons they prefer habitats with a mean basal area of 21.4 $m^2$/km and 17.2 $m^2$/km, respectively (Schroeder 1983). This species has been reported to forage on a variety of tree species including elms (Ulmus spp. Kisiel 1972), and paper birchs (*Betula paperifera*), infected with the ciccid (*Xylicocchus betulae*; Kilham 1970). They also were reported to feed extensively on pine bark beetles (*Dendroctonus pondersae*) during a severe winter outbreak of this species in Colorado (Crockett and Hansley 1978). They have been observed feeding on gall flies (*Eurosta solidagines*) along the border of woodlands (Schlichter 1978), and in corn stubble during the winter months in Illinois (Graber *et al.* 1977). Based on these studies, the downy woodpecker appears to be an opportunistic feeder utilizing whatever tree or other plant species provides an abundant food source.

Previous studies have suggested differences in feeding niches between male and female woodpeckers, thereby, reducing competition between the sexes (Rand 1952). In 1966, Selander reported the existence of sexual divergence in foraging behavior in two melanerpine woodpeckers, the insular Hispanitan woodpecker (*Melanerpes striatus*) and the continental golden-fronted woodpecker (*M. aurifrons*). Both Selander (1966) and Wallace (1974) suggested that sexual divergence of niche use would be reduced in continental communities because of the competition resulting from the diversity of the avifaunas. In the past two decades since Selander's (1966) initial paper, numerous studies have demonstrated the existence of sexual differences in foraging behavior in continental populations of Piciodae (Jackson 1970, Kilham 1970, Willson 1970, Kisiel 1972, Grubb 1975, Williams 1975, 1980). Rand (1952) and Selander (1966) suggest that differences in foraging behavior may be related to sexual dimorphism, but foraging differences are not always associated with morphological divergence (Ligon 1968, Jackson 1970, Kisiel 1972).

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For example, Williams (1975) found that male downy woodpeckers foraged on small branches while females probed bark crevices on trunks and larger branches. The current study examined habitat selection and foraging strategies of male and female downy woodpeckers in small woodlots adjacent in old field communities in western Pennsylvania and central Ohio.

## METHODS

Three woodlots were selected as study areas, two in western Pennsylvania and one in central Ohio at the Denison University Biological Reserve located near Granville, Ohio. The two sites in western Pennsylvania were located 16 km northeast of Grove City and 3 km west of New Wilmington, Pennsylvania, respectively. All three study areas were between 15 and 20 ha and were bordered by old field communities and cultivated lands.

The observer traveled a predetermined transect stopping at 10 minute intervals and viewing the surrounding area with either 7 x 35 or 8 x 50 binoculars. Vocalizations were also used to locate individual birds. During site visits, the observer identified the location of all downy woodpeckers observed on a grid map of the study area, along with the sex, behavior, location in the tree (trunk, large branches or small branches), and the tree or plant species in which the bird occurred. Each study area was visited once a week from 1 September 1990 through 30 April 1991 for a total of 32 visits/site and a total of over 300 hours of observations.

The number of birds observed in each habitat type was compared by a t distribution analysis and a chi square analysis was used for categorical comparisons of feeding preferences of male and female downy woodpeckers.

## RESULTS AND DISCUSSION

On all three study areas, downy woodpeckers appeared to prefer ecotone areas over forest habitat. Of the 350 individual observations of downy woodpeckers, 216 birds or 61.7% were observed in ecotone habitats compared to 134 or 38.2% found in forest habitats indicating that the species preferred ecotones over forested area ($t = 2.25$, $P < 0.05$). Moreover, downy woodpeckers used ecotone areas with a mixture of forest-shrub and grasslands more than either forest or forest-shrub habitats (Figure 1). There was no apparent difference between male and female downy woodpeckers using these different habitats.

The Habitat Suitability Index (HSI) developed for the downy woodpecker by Schroeder (1983) suggested that optimal foraging habitats for the species are those with a basal area between 10 and 20 $m^2$/km (43.6 and 87.2 $ft^2$/acre) and that stands with basal areas greater than 30 $m^2$/km (130.8 $ft^2$/acre) are assumed to have moderate values for downy woodpeckers. The ecotone areas on all study sites had lower basal areas than the woodlots which may explain their greater use by foraging birds.

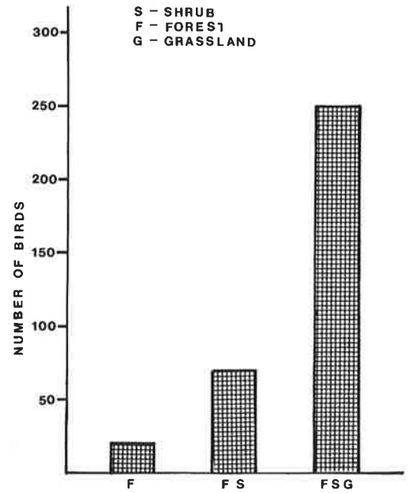


FIGURE 1. Number of downy woodpeckers (*Picoides pubescens*) observed in different habitats on three woodlots in Western Pennsylvania and Central Ohio.

Both male and female downy woodpeckers appear to be opportunistic feeders using a variety of species of plants as source of animal foods. During the study, birds were observed feeding on members of the following genera: *Ulmus, Acer, Carya, Quercus, Juglans, Fraxinus, Robinia, Fagus, Tilia, Prunus, Platunus, Catalpa, Salix,* and *Betula*. In addition, birds fed extensively on the fruit of poison ivy (*Rhus radicans*) which was common in all three study areas.

Male and female downy woodpeckers exhibited different feeding strategies on all three study areas. For the purpose of the study, a tree was considered to have three microhabitats available to the birds: trunk, large branches and small side branches (Figure 2). Males preferred to feed on smaller branches whereas female foraged primarily on the

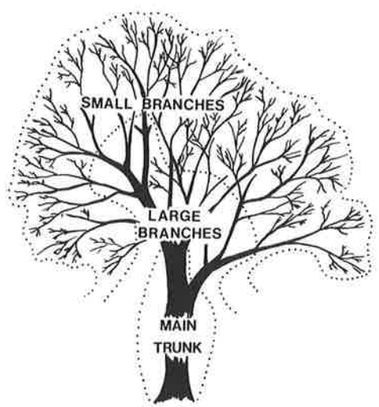


FIGURE 2. Three foraging microhabitats of the downy woodpecker (*Picoides pubescens*) in a deciduous tree.



trunk and large branches. Of the 246 feeding males, 143 (58.1%) were observed foraging on the smaller branches which differed significantly from the 23 or (9.3%) individuals observed foraging on the trunk or the 80 (32.5%) birds observed foraging on the larger branches ($x^2 = 27.7$, $P < 0.01$), respectively. Alternatively, of the 104 feeding females, 54 (51.9%) birds were observed foraging on the main trunk which was significantly greater than the 36 (34.6%) and the 14 (13.5%) birds observed foraging on large and small branches ($x^2 = 65.8$, $P < 0.01$), respectively.

Observations of the feeding behavior of individual birds revealed similar differences in foraging behavior. Based on 129 minutes of observations of 104 feeding sequences, females foraged 52% or 64.5 minutes compared to 38.5% or 49.7 minutes an 11.5% or 14.8 minutes on the large and small branches ($x^2 = 65.8$, $P < 0.01$), respectively. By comparison of the 151 minutes of observation of 180 feeding sequences, males spent 2.6 minutes (1.7%) foraging on the main trunk compared to 62.3 minutes (41.7%) and 85.1 minutes or (56.7%) on the large and small branches ($x^2 = 27.7$, $P < 0.001$), respectively. Male downy woodpecker foraged on small branches in greater frequency than would be expected if the birds were feeding randomly whereas females indicated a preference for trunks. Both sexes randomly foraged on the larger branches (Tables 1 and 2).

TABLE 1. Comparison of observed and expected foraging locations of male and female woodpeckers (*Picoides pubescens*) on three woodlots in Western Pennsylvania and Central Ohio.

| | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | Obs. | Exp. | Obs. | Exp. | Obs. | Exp. |
| Main Trunk | 23 | 54.1 | 54 | 22.8 | 77 | 76.9 |
| Large Branches | 80 | 81.5 | 36 | 34.5 | 116 | 116.0 |
| Small Branches | 143 | 110.3 | 14 | 46.7 | 157 | 157.0 |
| **Total** | **246** | **245.9** | **104** | **104.0** | **350** | **349.9** |

$x^2$ 27.7 $P < 0.001$ $x^2$ 65.8 $P < 0.001$

TABLE 2. Comparison of observed and expected time male and female woody woodpeckers (*Picoides pubescens*) spend foraging in different microbitats on three woodlots in Western Pennsylvania and Central Ohio.

| | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | Obs. | Exp. | Obs. | Exp. | Obs. | Exp. |
| Main Trunk | 3.6 | 36.7 | 64.5 | 31.4 | 68.1 | 68.1 |
| Large Branches | 62.3 | 60.4 | 49.7 | 51.6 | 112.0 | 112.0 |
| Small Branches | 85.1 | 53.9 | 14.8 | 46.0 | 99.9 | 99.9 |
| **Total** | **151.0** | **151.0** | **129.0** | **129.0** | **280.0** | **280.0** |

$x^2$ 48.0 $P < 0.001$ $x^2$ 129.1 $P < 0.001$

In conclusion, male and female downy woodpeckers prefer ecotones over forested areas, but the species exhibits a sexual divergence in foraging behavior. These differences in foraging behavior between male and female downy woodpeckers are similar to those found by Williams (1980) and support the hypothesis of Rand (1952) and Selander (1966) that sexual divergence in feeding behavior allows for a greater exploitation of available food resources while reducing competition between male and female downy woodpeckers.

## LITERATURE CITED

Beal, F. 1911. Food of the woodpeckers of the United States. U.S. Dept. Agric. Biol. Surv. Bull. 37:1-64.

Bent, A.C. 1939. Life histories of North American woodpeckers. U.S. Natl. Mus. Bull. 174. 334 pp.

Conner, R.N. 1980. Foraging habitats of woodpeckers in southwestern Virginia. J. Field Ornithol. 51:119-127.

Conner, R.N. 1981. Seasonal changes in woodpecker foraging patterns. Auk. 93:562-570.

Crockett, A.B. and P.L. Hansley. 1978. Apparent response of *Picoides* woodpeckers to outbreaks of pine bark beetle. Western Birds. 92:67-70.

Graber, J.W., R.R. Graber and E.L. Kirk. 1977. Illinois birds: Picoidae. Ill. Nat. Hist. Surv. Biol. Notes. 102:15-21.

Grubb, T.C. 1975. Weather-dependent foraging behavior of some birds wintering in a deciduous woodland. Condor 77:175-182.

Jackson, J.A. 1970. A quantitative study of the foraging ecology of downy woodpeckers. Ecology 51:318-323.

Jenkins, J.M. 1979. Foraging behavior of male and female nuttal woodpeckers. Auk 96:418-420.

Kilham, L.K. 1970. Feeding behavior of downy woodpeckers. 1. Preference for paper birches and sexual differences. Auk 87:544-556.

Kisiel, D. 1972. Foraging behavior of *Dendrocopos villosus* and *D. pubescens* in eastern New York state. Condor 74:393-398.

Ligon, D. 1968. Sexual differences in foraging behavior in two species of *Dendrocopos* woodpeckers. Auk 85:203-215.

Rand, A.L. 1952. Secondary sexual characters and ecological competition. Fieldiana Zool. 34:65-70.

Schlichter, L. 1978. Winter predation by black-capped chickadees and downy woodpeckers on inhabitants of the goldenrod ball gull. Gen. Field Nat. 92:71-74.

Schroeder, R.L. 1983. Habitat suitability index model: Downy woodpecker. U.S. Depart. Interior, Fish and Wildlife Service. 11 pp.

Selander, R.K. 1966. Sexual dimorphism and differential niche utilization in birds. Condor 68:113-151.

Wallace, R.A. 1974. Ecological and social complications of sexual dimorphism in five melanerpine woodpeckers. Condor 76:238-248.

Williams, J.B. 1975. Habitat utilization by four species of woodpeckers in a central Illinois woodland. Am. Midl. Nat. 93:354-367.

Williams, J.B. 1980. Intersexual niche partitioning in downy woodpeckers. Wilson. Bull. 92:439-451.

Willson, M.F. 1970. Foraging behavior of some winter birds of deciduous woods. Condor 72:129-132.

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# DUAL THERAPY IN THE TREATMENT OF GONORRHEA AND ITS EFFECT ON THE PREVALENCE OF *CHLAMYDIA TRACHOMATIS* INFECTION

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## ABSTRACT

**In 1985 the incidence of gonorrhea and chlamydia was determined in three independent health department STD clinic populations. Two health departments instituted a dual treatment regimen (ceftriaxone and doxycycline) for all patients diagnosed with gonorrhea. The other health department continued a single treatment regimen and utilized screening, contact notification and referral as a chlamydial control strategy. In 1990, the incidence of gonorrhea and chlamydia was again determined in the three populations to evaluate the efficacy of dual therapy with respect to chlamydial control. From 1985 to 1990, there was a decrease in the number of cases of gonorrhea and chlamydia in the two health departments that used the dual treatment regimen. After five years of screening, contact notification and referral and a single treatment regimen the third health department showed an increase in the number of gonorrheal and chlamydial cases.**

**These data suggest that dual therapy alone may have contributed to a significant reduction in diagnosed chlamydial as well as gonorrheal cases. A control effort combining both screening, contact notification and referral, and dual therapy might result in even greater reduction in chlamydial morbidity. [J PA Acad Sci 66(1):18-20, 1992]**

## INTRODUCTION

Infections caused by *Chlamydia trachomatis* are now recognized as the most prevalent and damaging of all sexually transmitted diseases (STD). An estimated 4 million people suffer from a chlamydial infection each year (Washington *et al.* 1986). Men, women and infants are affected but women bear an inordinate burden because of their increased risk for adverse reproductive consequences (Centers for Disease Control, CDC 1985). Chlamydia accounts for one-quarter to one-half of the 1 million recognized cases of PID in the United States each year. These infections contribute significantly to the increasing number of women who experience ectopic pregnancy or involuntary infertility. (Henry-Suchet 1988, Chow *et al.* 1990). Chlamydial infections of the cervix of prenatal women (Much and Yeh 1991) may result in infant pneumonia and conjunctivitis (Schachter *et al.* 1986). Enormous cost is associated with chlamydial infections. Each year, more than $1 billion in direct and indirect costs are expended on these infections in the United States (Washington *et al.* 1987). Chlamydial infections have emerged as one of the most important women's health problems.

Many local health departments find it increasingly difficult to keep up with the cost of locating, screening and treating the increasing number of chlamydial cases, especially the 70% of women with endocervical infection that are asymptomatic (CDC 1985). Because concomitant chlamydial and gonorrheal infection rates are high [15 - 20% in men; 25 - 60% in women] (CDC 1985, Stamm *et al.* 1984, Csango *et al.* 1984, Brunham *et al.* 1982, Much 1987), it has been suggested by the Centers for Disease Control, CDC (1985), that gonorrheal infection is an epidemiologic marker for the presumptive diagnosis of chlamydial infection. The CDC has therefore recommended the combination of ampicillin and tetracycline as the regimen of choice for uncomplicated gonorrheal infection in adults. Our study suggests that the treating of all positive gonorrheal patients with ceftriaxone and doxycycline is an effective method of decreasing the prevalence of chlamydia and gonorrhea in the same population.

## MATERIALS AND METHODS

Approximately 75,000 patients who presented to the STD clinic of three independent health departments were examined for gonorrhea and chlamydia. The number of positive cases reported in 1985 was compared to the number of positive cases in 1990. The health departments were Allentown Health Bureau, Allentown, PA; Allegheny County Health Department, Pittsburgh, PA; and King County Health Department, Seattle, WA. Each department utilized an immunoassay procedure to determine the presence of *Chlamydia trachomatis*, and gram stain and culture to detect the presence of *Neisseria gonorrheae*, in urethral and cervical smears. Single therapy regimen consisted of one injection of ceftriaxone (250 mg IM) (or a CDC recommended alternative) to treat gonorrhea, and doxycycline (100 mg, twice a day for 7 days) to treat chlamydia. Dual therapy consisted of the administration of both antibiotics to patients positive for gonorrhea.

## RESULTS

The Allentown Health Bureau and the King County Health Department instituted dual therapy in 1985 as a treatment for gonorrhea. The Allegheny County Health Department treated these two infections separately. The number of patients positive for gonorrhea, chlamydia or co-infected with both bacteria decreased over a five year period from 1985 to 1990 when dual therapy was used to treat positive gonorrheal patients; but, increased when a single therapy regimen was employed (Table 1).

TABLE 1. Percent change in diagnosed cases of gonorrhea and chlamydia: 1985 to 1990.

| | Gonorrhea | Chlamydia | Co-Infected |
|---|---|---|---|
| Allentown Health Bureau | −2% | −15% | −10% |
| King County Health Dept. | −44% | −5% | −43% |
| Allegheny County Health Dept. | +38% | +11% | +50% |

## DISCUSSION

The rate of co-infection with chlamydia in patients diagnosed with gonorrhea ranges from 15 to 20 percent in males and 25 to 60 percent in females (CD 1985, Stamm *et al.* 1984, Csango *et al.* 1984, Brunham *et al.* 1982, Much 1987). Therefore a significant number of chlamydial infections will be treated when co-therapy is used to treat patients positive for gonorrhea. Previous investigators have demonstrated the efficacy (Stamm *et al.* 1984, Csango *et al.* 1984, Brunham *et al.* 1982) and cost-effectiveness (Washington *et al.* 1987) of combined therapy. In Allengheny County the increase in both chlamydial and gonorrheal infections may have been due to the use of a single treatment regimen and the resultant increased probability of treatment failures. Furthermore, by treating all patients positive for gonorrhea with ceftriaxone and with a seven day course of doxycycline, the period of communicability is reduced by at least one week. This would interrupt the chain of infection by preventing both further transmission of chlamydia and reinfection with gonorrhea. In this way a co-infected patient diagnosed with gonorrhea and treated with both antibiotics is prevented from transmitting and acquiring chlamydia for one week which is ample time for sexual contacts to be notified and referred for examination and treatment. In addition the use of dual therapy would decrease the treatment failure rate for gonorrhea.

Combined therapy is an effective means of reducing the prevalence of both gonorrhea and chlamydia. However, this regimen does not obviate the need for laboratory diagnosis and epidemiologic contact notification and referral. Such a protocol is important for clinical diagnosis, education of patients and physicians, therapeutic compliance, referral of sexual partners, determining high risk groups, as well as the detection of unsuspected chlamydial infections (Stamm *et al.* 1986, Handsfield *et al.* 1986, Handsfield 1987.) Therefore, combined therapy should be an additional stage in the surveillance and control of the chlamydial epidemic.

## LITERATURE CITED

Brunham, R.C., C.C. Kuo and W.J. Chen. 1982. Treatment of concomitant *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections in women: Comparison of trimethoprimsulfamethoxazole with ampicillin-probenecid. Rev. Infect. Dis. 4:491-494.

Centers for Disease Control (CDC). 1985. *Chlamydia trachomatis* Infections. Policy Guidelines for Prevention and Control. Morbidity and Mortality Weekly Report. 34 (Suppl. 3S): iiis pp 53S-74S.

Chow, J.M., M.L. Yonekura, G.A. Richwald, S. Greenland, R.L. Sweet and J. Schachter. 1990. The association between *Chlamydia trachomatis* and ectopic pregnancy. J. Amer. Med. Assoc. 263:3164-3167.

Csango, P.A., A. Salveson, T. Gundersen, G. Jagers and O. Bjerk. 1984. Treatment of acute gonococcal urethritis in men with simultaneous infection with *Chlamydia trachomatis.* Br. J. Vener. Dis. 60:95-98.

Handsfield, H.H., L.L. Jasman, P.L. Roberts, V.W. Hanson, R.L. Kothenbeutel and W.E. Stamm. 1986. Criteria for selective screening for *Chlamydia trachomatis* infection in women attending family planning clinics. J. Amer. Med. Assoc. 255:1730-1734.

Handsfield, H.H. 1987. Control of sexually transmitted chlamydial infections. J. Amer. Med. Assoc. 257:2073-2074.

Henry-Suchet, J. 1988. *Chlamydia trachomatis* infection and infertility in women. J. Reprod Med. 33:912-916.

Much, D.H. and S. Yeh. 1991. Prevalence of *Chlamydia trachomatis* in pregnant patients. Public Health Reports. 106:490-493.

Much, D.H. 1987. A comparative study of *Neisseria gonorrhoeae, Chlamydia trachomatis* and *Ureaplasma urealyticium* isolated from a sexually active population. J. Pa. Acad. Sci. 61:41-46.

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Schachter, J. M. Grossman, R.L. Sweet, J. Holt and E. Bishop. 1986. Prosepective study of perinatal transmission of *Chlamydia trachomatis*. J. Am. Med. Assoc. 27:3374-3377.

Stamm, W.E. and K.K. Holmes. 1986. Measures to control *Chlamydia trachomatis* infections: An assessment of new national policy guidelines. J. Amer. Med. Assoc. 256:1178-1179.

Stamm, W.E., M.E. Guinan, C. Johnson, T. Starcher, K.K. Holmes and W.M. McCormack. 1984. Effect of treatment regimens for *Neisseria gonorrhoeae* on simultaneous infection with *Chlamydia trachomatis*. N. Eng. J. Med. 310:545-549.

Washington, A.E., R.E. Johnson and L.L. Sanders. 1987. *Chlamydia trachomatis* infections in the United States. What are they costing us? J. Amer. Med. Assoc. 257:2070-72.

Washington, A.E., R.E. Johnson and L.L. Sanders. 1986. Incidence of *Chlamydia trachomatis* infections in the United States: Using reported *Neisseria gonorrhoeae* as a surrogate, in Oriel J.D. *et al.* (eds): *Chlamydia trachomatis* infections: Procedures of the sixth international symposium on human *Chlamydia trachomatis* infections. New York, Cambridge University Press, pp. 487-490.

Washington, A.E., W.S. Browner and C.C. Korenbrot. 1987. Cost-effectiveness of combined treatment for endocervical gonorrhoeae. Considering co-infection with *Chlamydia trachomatis*. J. Amer. Med. Assoc. 257:2056-2060.

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# AN INVESTIGATION INTO THE BIOISOSTERISM OF VINYL AND CYCLOPROPYL GROUPS IN LOCAL ANESTHETICS[1]

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## ABSTRACT

**Several series of N,N-dialkylaminoethyl esters of cinnamic acid, 1-phenylcyclopropane carboxylic acid and trans 2-phenylcyclopropane carboxylic acid were synthesized. The 1-phenylcyclopropane carboxylates were made using 1-phenylcyclopropane nitrile as a key intermediate while the 2-phenylcyclopropane carboxylates were synthesized from styrene by a rhodium acetate catalyzed cyclopropanation. The compounds were then examined for local anesthetic activity using a goldfish overturn assay and for acetylcholinesterase inhibition by an *in vitro* enzyme assay. The relative order of activity was found to be: cinnamic acid esters ≃ 2-phenylcyclopropane carboxylate esters > 1-phenylcyclopropane carboxylate esters. In all three series of compounds a p-chloro substituent appeared to provide the greatest potentiation of activity. It was also shown that neither of the cyclopropane derivatives were able to provide conjugation of the aromatic ring with the carboxylate carbonyl group. Thus, although the cyclopropane ring appears to be bioisosteric with the vinylic double bond, the bioactivity is not dependant on the conjugative ability of the cyclopropane ring system.**
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## INTRODUCTION

A substantial body of literature exists which supports the theory that cyclopropane and its derivatives are unique among the cycloalkanes because of the olefinic character of their C-C bonds. Cyclopropane has been labeled as the saturated analog of a carbon-carbon double bond. Coulson and Moffit's model (1949) of cyclopropane consists of bent bonds in which the maximum overlap of the carbon orbitals is permitted when the carbons are nearly $sp^5$ hybridized. Pews and Ojha (1969) have shown that the cyclopropane ring is intermediate between a dimethylene group ($-CH_2CH_2-$) and a vinylic group ($-CH=CH-$) in its ability to transmit conjugation and have attributed this conjugation to the overlap of an aromatic $\pi$ system with the enriched p orbital character of the bent bonds of the cyclopropane ring. Bernett (1967) has provided a unified theory for the unique bonding in the cyclopropane ring.

Previous evidence suggests that there is also a bioisosteric relationship between a cyclopropane ring and a vinylic double bond in opiate analgesics, (Loew and Berkowitz, 1978) spirolactone mineralocorticoids (Chinn, *et al.* 1981), lipoxygenase inhibitors (Arai, *et al.* 1983) and quinolone antibacterials (Domagela, *et al.* 1988).

Our interest in extending this relationship to local anesthetics was stimulated by the early work of Brill and Cook (1933) who demonstrated that diethylaminoethyl cinnamate had local anesthetic properties while its saturated dihydrocinnamate analog did not.

Galinsky *et al.* (1963) have shown, in a series of procaine derivatives, that the degree of conjugation of the carbonyl group with the aromatic ring correlated with the local anesthetic activity of these compounds.

Gerhards, *et al.* (1986) have recently performed a quantum mechanical analysis of the interaction between Acetylcaine and a model receptor. They propose that increasing the polarity of the carbonyl group of a local anesthetic may enhance the interaction with the receptor.

In view of these findings, it seemed to us that a suitable replacement of the carbon-carbon double bond in the cinnamic acid esters by a cyclopropane ring would provide a test of whether the enhanced p character of the cyclopropane ring was sufficient to provide conjugation and bioisosterism in ester type local anesthetics containing such a ring system. Galinsky *et al.* (1963) suggest that if nerve desensitization occurs by a competition of the drug with acetylcholine for an enzyme site, a change in the bond order of the carbonyl portion of the ester could influence this interaction and thus the local anesthetic activity.

We therefore decided to prepare three sets of compounds (Table 2) containing either a vinyl group or a cyclopropyl group in which the orientation of the cyclopropane ring

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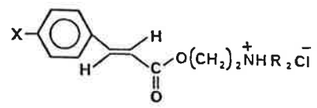
replacing the carbon to carbon double bond would be either edgebonded (1,2-disubstituted) or corner bonded (1,1-disubstituted). A search of the literature revealed that several aminoalkyl esters of 1-phenylcyclopropane carboxylic acid (the corner bonded analog) have been previously prepared and examined for antispasmodic activity (Weston, 1946), while several $\beta$-diethylaminoethyl esters of 2-aryl substitued cyclopropane carboxylic acid (the edge bonded analog) were prepared and evaluated for local anesthetic and antispasmodic activity (Burger, *et al.* 1949). In both cases low levels of activity were reported.

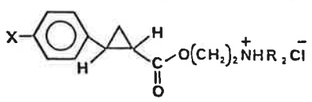
The currently accepted mechanism of action of local anesthetics involves a blockage of axonal conduction in post synaptic neural fibers. These drugs bind to a site in the sodium channel complex (McNeal, *et al.* 1985) thereby interfering with the influx of sodium ions during the early phase of the action potential (Hille, 1977). Evidence supports the theory that local anesthetics bind to (Blanchard, *et al.* 1979) and block (Neher and Steinbach, 1978) acetylcholine receptors located within the open ion channels in the membrane.

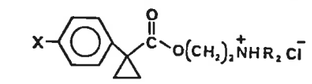
Fish, because of their anatomic structure, are particularly disposed to show a strong reaction to substances with a pharmacological action. When such a substance is dissolved in the water which serves the fish as medium and that substance passes through the mucous membranes of the gills, it will be transported directly to those organs which are sensitive to the substance, thereby inducing a physiological response. (Witjens, *et al.* 1947)

Several reports in the literature (Adams, *et al.* 1926 and Feldman, *et al.* 1975) suggest that a convenient screening for local anesthetic activity can be achieved using a goldfish assay where loss of the righting reflex is a definite, reproducible bioendpoint.

TABLE 2. N,N-Dialkylaminoethyl Cinnamates and Cyclopropyl Analogs







| Compd | X | R | Yield, %[a] | Recryst. Solv. | mp°C[b] | Formula | Anal.[c] |
|---|---|---|---|---|---|---|---|
| 1 | H | Me | 86 | MeOH/$Et_2O$ | 122-4 | $C_{13}H_{18}NO_2Cl$ | C,H |
| 2 | H | Et | 53 | EtOH | 153-5[e] | $C_{15}H_{22}NO_2Cl$ | C,H |
| 3 | H | i-Pr | 90 | MeOH | 121-2 | $C_{17}H_{26}NO_2Cl$ | C,H |
| 4 | Cl | Me | 75 | MeOH/$Et_2O$ | 187-9 | $C_{13}H_{17}NO_2Cl_2$ | C,H |
| 5 | MeO | Me | 78 | MeOH/$Et_2O$ | 154-6 | $C_{14}H_{20}NO_3Cl$ | C,H |
| 6 | H | Me | 83 | EtOAc | 111-3 | $C_{14}H_{20}NO_2Cl$ | C,H |
| 7 | H | Et | 12[d] | EtOAc | 115-7[f] | $C_{16}H_{24}NO_2Cl$ | C,H |
| 8 | Cl | Me | 80 | EtOAc | 124-6 | $C_{14}H_{19}NO_2Cl_2$ | C,H |
| 9 | MeO | Me | 91 | EtOAc | 130-1 | $C_{15}H_{22}NO_3Cl$ | C,H |
| 10 | H | Me | 88 | EtOAc | 131-2 | $C_{14}H_{20}NO_2Cl$ | C,H |
| 11 | Cl | Me | 89 | MeOH/$Et_2O$ | 173-4 | $C_{14}H_{19}NO_2Cl_2$ | C,H |
| 12 | MeO | Me | 95 | EtOAc | 146-8 | $C_{15}H_{22}NO_3Cl$ | C,H |
| 13 | MeO | Et | 26[d] | EtOAc | 153-5 | $C_{17}H_{26}NO_3Cl$ | C,H |

[a] Crude yield based on the carboxylic acid. Compounds were purified by recrystallization and/or column chromatography.

[b] uncorrected.

[c] Analyses indicated by elemental symbols were within ± 0.4% of the theoretical values. See appendix.

[d] Recrystallized recovery from crude product.

[e] Brill and Cook, 1933. mp 135°.

[f] Burger, *et al.* 1949. bp 161° (4.4 torr) for the free base.

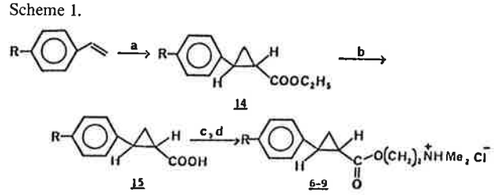
## METHODS AND MATERIALS

Melting points were measured on a Fisher Mel-temp apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer Model 137 spectrophotometer. The $^1$H-NMR spectra were recorded on either a Perkin-Elmer Model R-20B or a Varian EM-360A instrument using TMS as an internal standard. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN and were within ± 0.4% of the calculated values. The *trans*-cinnamic acids were commercially available from Aldrich Chemical Company and were used without further purification. The following were prepared by the method outlined in Scheme 1.

*Ethyl 2-Phenylcyclopropane Carboxylate* (*14*, R = H)

To a solution of 0.022 g (.05 mmol) of rhodium (II) acetate in 54.9 mL (0.48 mol) of deinhibited styrene was added 5.1 mL (0.048 mol) of ethyl diazoacetate dropwise over 3½ hours at room temperature under nitrogen. When an IR spectrum no longer showed a peak at 2170 cm$^{-1}$, the solution was shaken with water and the blue layer containing the catalyst was removed. The organic layer was dried over $MgSO_4$ and vacuum distilled. This yielded 5.92 g (0.031 mol, 65%) of a mixture of the *trans* and *cis* isomers of ethyl 2-phenylcyclopropane carboxylate, bp. 112-114° (3 torr). (Burger and Yost, 1948 bp 103-105° at 0.7 torr). $^1$H-NMR (neat): δ 7.15 (m,5H,ArH), 4.1 (q,1.26H, $-OCH_2-$ [trans]), 3.75 (q,0.74H, $-OCH_2-$ [cis]), 2.6-1.3 (m,4H,cyclopropyl H), 1.1 (t,1.9H,$CH_3$[trans]), 0.85 (t,1.1H,$CH_3$[cis]). From the ratio of the intensity of integration of the methyl and methylene signals from the 2 isomers, the trans/cis ratio was measured as 1.7.

Scheme 1.



a. $N_2CHCOOEt$, b. KOH/EtOH, c. $SOCl_2$, d. $HO(CH_2)_2NMe_2$, φH



*Ethyl 2-(p-Chlorophenyl) cyclopropane Carboxylate* (*14*, R = Cl)

Rhodium acetate (44 mg, 0.10 mmol) and 17.3 g (.125 mol) p-chlorostyrene were dissolved in 25 mL of dry benzene. The bright blue-green solution was cooled to 25° and vigorously stirred during a very slow addition (2-4 drops per minute) of 3.4 g (.030 mol) ethyl diazoacetate from a dropping funnel. The entire system was blanketed with $N_2$ throughout the addition. After the reaction was complete, the mixture was washed several times with water to remove the $Rh_2(OAc)_4$ and the benzene solution was dried over $MgSO_4$, filtered and the solvent removed on a rotovap.

The residual oil was then fractionally distilled at reduced pressure to yield a 4.1 g fraction (0.018 mol,61%) bp 155-158° (4 torr), which was identified as a trans/cis mixture of ethyl-2-(p-chlorophenyl) cyclopropane carboxylate. IR: 1730 cm$^{-1}$ (C = O). $^1$H-NMR (neat): δ 7.05 (m,4H,ArH), 4.1 (q,1.15H, $-OCH_2-$ [trans]), 3.8 (q,0.85H, $-OCH_2-$ [cis]), 2.6-1.3 (m,4H,cyclopropyl H), 1.1 (t,1.72H, $-CH_3$[trans]), 0.9 (t,1.28H, $-CH_3$[cis]). From the ratio of the intensity of integration of the methyl and methylene signals from the 2 isomers, the trans/cis ratio was measured as 1.35.

*Ethyl 2-(p-Methoxyphenyl) cyclopropane Carboxylate* (*14* R = $OCH_3$)

Prepared in a similar manner as above. bp 139-142° (2 torr). mp 80-82° (House, *et al.* 1978 mp 81-83° $^1$H-NMR ($CDCl_3$): δ 6.9 (dd,4H,ArH), 4.15 (q,2H, $-OCH_2-$ [trans]), 3.65 (s,3H,$CH_3O$), 2.4 (m,1H) and 1.9-1.3 (m,3H,cyclopropyl H), 1.2(t,3H,$CH_3$[trans]).

*trans 2-Phenylcyclopropane Carboxylic Acids* (*15*)

A solution of 2.0 g of each of the esters (*14*) and 1.0 g NaOH in 20 mL of ethanol and 10 mL $H_2O$ was refluxed for 24 hours. Acidification with 12 M HCl precipitated the free acid, which was recrystallized either from water or aqueous ethanol to give the pure trans-acids.

*trans 2-Phenylcyclopropane Carboxylic Acid* (*15*, R = H)

mp 87-9°. (Burger and Yost, 1948 mp 93°). $^1$H-NMR ($CCl_4$): δ 10.4 (s,1H,COOH), 7.15 (s,5H,ArH), 2.5 (m,1H) and 1.9-1.2 (m,3H,cyclopropyl H).

*trans 2-(p-Chlorophenyl) cyclopropane Carboxylic Acid* (*15*, R = Cl)

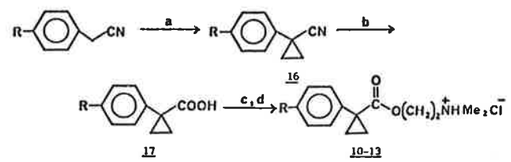
mp 114-116°. IR (KBr): ν 1680 cm$^{-1}$ (C = O). $^1$H-NMR ($CDCl_3$): δ 11.1 (s,1H,COOH), 7.1 (dd,4H,ArH), 2.5 (m, 1H) and 2.0-1.1 (m,3H,cyclopropyl H).

*trans 2-(p-Methoxyphenyl) cyclopropane Carboxylic Acid* (*15*, R = $OCH_3$)

mp 109-110°. (House, *et al.* 1978 mp 113-114°) $^1$H-NMR ($CDCl_3$): δ 10.4 (s,1H,COOH), 7.0 (dd,4H,ArH), 3.75 (s,3H,$CH_3O$), 2.5 (m, 1H) and 2.2-1.2 (m,3H,cyclopropyl H).

The following were prepared by the method outlined in Scheme 2.

Scheme 2.



a. 2BuLi, $Cl(CH_2)_2Cl$ b. $NaOH/H_2O$ c. $SOCl_2$ d. $HO(CH_2)_2NMe_2$, φH

*1-Phenylcyclopropane Nitrile* (*16*, R = H)

Prepared using the procedure of Kaiser. bp 83-84° (0.85 torr); (Kaiser, *et al.* 1971 bp 89-90.5° at 2.5 torr) IR (neat): ν 2220 cm$^{-1}$ (CN)$^1$H-NMR (neat): δ 7.2 (s,5H,ArH), 1.4 and 1.15 (m,4H,cyclopropyl H).

*1-(p-Methoxyphenyl) cyclopropane Nitrile* (*16*, R = $OCH_3$)

n-Butyl lithium (225 mL of a 1.6 M solution, 0.36 mol) was rapidly added to 224 mL of freshly distilled THF. Reflux, with stirring, was established. From an attached pressure-equalized dropping funnel 23.5 g (0.16 mol) p-methoxyphenyl acetonitrile diluted in 160 mL THF was added over 5 minutes. The mixture was stirred for 1 hour during which the solution darkened to a transparent brown. From the dropping funnel 17.0 g (0.17 mol) 1,2-dichloroethane in 300 mL THF was then added to the flask over 60 minutes. The resulting solution was refluxed under nitrogen for 16 hours, cooled and acidified with 320 mL of 3 N HCl. The organic layer was separated, dried over $MgSO_4$, filtered and distilled to remove the THF solvent. The residual oil was then vacuum distilled to yield 16.4 g (0.094 mol, 59%) of product, bp 124-130° (0.9 torr). IR (neat): ν 2240 cm$^{-1}$ (CN). $^1$H-NMR ($CCl_4$): δ 6.9 (dd,4H, ArH), 3.7 (s,3H,$CH_3O$-), 1.5 and 1.25 (m,4H,cyclopropyl H).

*1-Phenylcyclopropane Carboxylic Acid (17,* R = H)

A mixture of 5.0 g (.035 mol) 1-phenylcyclopropane nitrile and 20 mL of 20% aqueous NaOH was refluxed with stirring for 24 hours. By the end of that period a homogeneous solution resulted. The reaction mixture was added to 150 mL of water and the clear solution was acidified to precipitate 5.0 g (0.031 mol, 89%) of free acid, mp 84-6° (Knowles and Cloke 1932 mp 86-87°). IR (KBr): ν 1695 cm$^{-1}$ (C = O). $^1$H-NMR ($CDCl_3$): δ 12.3 (s,1H,COOH), 7.2 (s,5H,ArH), 1.6 and 1.2 (m,4H,cyclopropyl H).

*1-(p-Methoxyphenyl) cyclopropane Carboxylic Acid* (*17,* R = $OCH_3$)

mp 150-152° was obtained from the Aldrich Chemical Company, Milwaukee, WI, and used as received,

*1-(p-Methoxyphenyl) cyclopropane Carboxylic Acid* (*17,* R = $OCH_3$)

Prepared as above. mp 130-131.5°. IR (nujol): ν 3200-2400 cm$^{-1}$ (COOH). $^1$H-NMR ($CCl_4$): δ 11.9 (s,1H,COOH), 6.9 (dd,4H,ArH), 3.7 (s,3H,$CH_3O$-), 1.6 and 1.2 (m,4H,cyclopropyl H).

*N,N-Dialkylaminoethyl Cinnamates (1-5) and N,N-Dialkylaminoethyl 1-Phenylcyclopropane Carboxylates (10-13)* (Table 2)

The cinnamic acid or 1-phenylcyclopropane carboxylic acid (0.050 mol) was mixed with thionyl chloride (.10 mol) and a drop of DMF. The mixture was allowed to react in a warm water bath for ½ hour. The excess $SOCl_2$ was removed by vacuum distillation and azeotropic distillation with low boiling petroleum ether. Upon complete removal of the excess $SOCl_2$, the acid chloride was obtained. IR confirmed complete conversion to the acid chloride. This was not purified further but was directly reacted with the N,N-dialkylaminoethanol as follows:

The acid chloride was dissolved in 125 mL of dry benzene and to this stirred solution was added a solution of 0.040 mol of freshly distilled N,N-dialkylaminoethanol in 50 mL of benzene dropwise over 15-30 minutes. The solution clouded almost immediately and within a few minutes, a white solid began to form. Stirring was continued overnight. The white solid was filtered with suction and washed extensively with dry benzene to yield the crude product as its hydrochloride salt.

Recrystallization was accomplished either from ethyl acetate or from a methanol/diethyl ether mixture. Column chromatography, when used, was done on silica gel using a 10% MeOH in $CHCl_3$ eluant. The yields and melting points of these products are given in Table 2. They were characterized by TLC on silica gel plates, $^1$H-NMR and elemental analysis.

*N,N-Dimethylaminoethyl Cinnamate HCl (1)*

$^1$H-NMR ($D_2O$): δ 7.5 (d, 1H) and 6.5 (d,1H,vinylic H), 7.25 (s,5H,ArH), 4.7 (HDO), 4.35 (m,2H, $-OCH_2-$), 3.4 (m,2H, $-NCH_2-$), 2.85 (s,6H,$NCH_3$).

*N,N-Diethylaminoethyl Cinnamate HCl (2)*

$^1$H-NMR ($D_2O$): δ 7.6 (d, 1H) and 6.4 (d,1H,vinylic H), 7.45 (s,5H,ArH), 4.7 (HDO), 4.5 (m,2H, $-OCH_2-$), 3.4 (m,2H, $-NCH_2-$), 3.25 (q,4H, $-CH_2-$), 1.25 (t,6H, $-CH_3$).

*N,N-Diisopropylaminoethyl Cinnamate HCl (3)*

$^1$H-NMR ($D_2O$): δ 7.5 (d, 1H) and 6.3 (d,1H,vinylic H), 7.35 (s,5H,ArH), 4.7 (HDO), 4.4 (m,2H, $-OCH_2-$), 3.65 (heptet,2H,CH), 3.3 (m,2H, $-NCH_2-$), 1.25 (d,12H,$(CH_3)_2$).

*N,N-Dimethylaminoethyl p-Chlorocinnamate HCl (4)*

$^1$H-NMR ($D_2O$): δ 7.5 (d,1H) and 6.3 (d,1H,vinylic H), 7.2 (dd,4H,ArH), 4.7 (HDO), 4.5 (m,2H, $-OCH_2-$), 3.55 (m,2H, $-NCH_2-$), 2.95 (s,6H,$NCH_3$).

*N,N-Dimethylaminoethyl p-Methoxycinnamate HCl (5)*

$^1$H-NMR ($D_2O$): δ 7.5 (d, 1H) and 6.2 (d,1H,vinylic H), 7.05 (dd,4H,ArH), 4.7 (HDO), 4.5 (m,2H, $-OCH_2-$), 3.65 (s,3H,$CH_3O$), 3.5 (m,2H, $-NCH_2-$), 2.95 (s,6H,$NCH_3$)

*N,N-Dimethylaminoethyl-1-Phenylcyclopropane Carboxylate HCl (10)*

$^1$H-NMR ($D_2O$): δ 7.4 (s,5H,ArH), 4.7 (HDO), 4.4 (m,2H, $-OCH_2-$), 3.4 (m,2H, $-NCH_2-$), 2.7 (s,6H,$NCH_3$), 1.65 and 1.35 (m,4H,cyclopropyl H).

*N,N-Dimethylaminoethyl-1-(p-chlorophenyl) cyclopropane Carboxylate HCl (11)*

$^1$H-NMR ($D_2O$): δ 7.35 (s,4H,ArH), 4.7 (HDO), 4.4 (m,2H, $-OCH_2-$), 3.4 (m,2H, $-NCH_2-$), 2.75 (s,6H,$NCH_3$), 1.65 and 1.3 (m,4H,cyclopropyl H).

*N,N-Dimethylaminoethyl-1-(p-methoxyphenyl) cyclopropane Carboxylate HCl (12)*

$^1$H-NMR ($D_2O$): δ 7.0 (dd,4H,ArH), 4.55 (HDO), 4.25 (m,2H, $-OCH_2-$), 3.7 (s,3H,$CH_3O$), 3.25 (m,2H, $-NCH_2-$), 2.65 (s,6H,$NCH_3$), 1.5 and 1.15 (m,4H, cyclopropyl H).

*N,N-Diethylaminoethyl-1-(p-methoxyphenyl) cyclopropane Carboxylate HCl (13)*

$^1$H-NMR ($D_2O$): δ 7.1 (dd,4H,ArH), 4.6 (HDO), 4.3 (m,2H, $-OCH_2-$), 3.7 (s,3H,$CH_3O$), 3.3 (m,2H, $-NCH_2-$), 2.9 (q,4H, $-CH_2-$), 1.5 and 1.25 (m,4H,cyclopropyl H), 1.0 (t,6H, $-CH_3$).

*N,N-Dialkylaminoethyl trans-2-Phenylcyclopropane Carboxylates (6-9)* (Table 2)

Reaction conditions were the same as above except that the hydrochloride salts of the 2-phenylcyclopropane carboxylates were hygroscopic and thus had to be isolated in a nitrogen atmosphere and dried under vacuum. Once thoroughly dried their hygroscopic behavior was not nearly so troublesome.

*N,N-Dimethylaminoethyl trans-2-Phenylcyclopropane Carboxylate HCl (6)*

$^1$H-NMR ($D_2O$): δ 7.25 (m,5H,ArH), 4.7 (HDO), 4.45 (m,2H, $-OCH_2-$), 3.45 (m,2H, $-NCH_2-$), 2.9 (s,6H,$NCH_3$), 2.6 (m,1H) and 2.1-1.4 (m,3H,cyclopropyl H).

*N,N-Diethylaminoethyl trans-2-Phenylcyclopropane Carboxylate HCl (7)*

$^1$H-NMR ($D_2O$): δ 7.25 (m,5H,ArH), 4.7 (HDO), 4.4 (m,2H, $-OCH_2-$), 3.4 (m,2H, $-NCH_2-$), 3.25 (q,4H, $-CH_2-$), 2.5 and 2.1-1.2 (m,4H,cyclopropyl H), 1.25 (t,6H, $-CH_3$).

*N,N-Dimethylaminoethyl trans-2(p-chlorophenyl) cyclopropane Carboxylate HCl (8)*

$^1$H-NMR ($CDCl_3$): δ 7.1 (dd,4H,ArH), 4.55 (m,2H, $-OCH_2-$), 3.4 (m,2H, $-NCH_2-$), 2.9 (d,6H, $NCH_3$), 2.5 (m, 1H) and 2.0-1.2 (m,3H,cyclopropyl H).



*N,N-Dimethylaminoethyl trans-2 (p-methoxyphenyl) cyclopropane Carboxylate HCl (9)*

$^1$H-NMR ($D_2O$): δ 7.05 (dd,4H,ArH), 4.7 (HDO), 4.5 (m,2H, $-OCH_2-$), 3.8 (s,3H,$CH_3O$), 3.55 (m,2H, $-NCH_2-$), 2.95 (s,6H,$NCH_3$), 2.5 (m, 1H) and 2.1-1.4 (m,3H,cyclopropyl H).

*Goldfish Overturn Assay*

All testing was done at 20° ± 1°C using common Goldfish (2-3g) between 1-6 hours after feeding. The pH of all test solutions fell between 6.6-7.0. The test solutions were made up to a 3.0 mM concentration using dechlorinated tap water and were freshly prepared before each set of determinations. Three fish were introduced into 500 mL of the test solution contained in a 1 L beaker at time zero. The response time was taken as that point where the goldfish lost its balance and turned over unto its side. The determination was repeated until at least six reproducible times were measured.

*Acetylcholinesterase Inhibition Assay*

For the biochemical assay, all chemicals were reagent grade. The acetylcholine chloride, acetylcholinesterase from electric eel, and the 0.10 M pH 7.5 TRIS buffer components were obtained from Sigma Chemical Company. The freeze dried acetylcholinesterase was reconstituted with TRIS buffer and diluted to obtain an activity of 0.12 μM acetylcholine hydrolyzed per mL per minute. Optical densities were measured on a Beckman Model DBG Spectrophotometer. An Orion Research digital pH meter was used for all pH measurements.

The concentration of acetylcholine was measured by the colorimetric method of Hestrin (1949). Alkaline hydroxylamine reagent was freshly prepared from equal volumes of 2 M hydroxylamine hydrochloride and 3.5 M sodium hydroxide solutions. Four mL of this reagent were mixed with 2.0 mL of acetylcholine solution. After one minute, the mixture was acidified with 33.3% HCl and 2.0 mL of 0.37 M $FeCl_3$ solution was added to develop color. Then the optical density was measured at 540 nm.

In order to determine the inhibitory properties of the local anesthetics, they were added in varying amounts to a specified amount of acetylcholine in TRIS buffer and allowed to compete for reaction with the enzyme at 25°C. The acetylcholine concentration (5.0 mM), the amount of the enzyme (0.20 mL) and the reaction time were kept constant. The time chosen was such that the amount of acetylcholine hydrolyzed was less than 50%. After 25 minutes, the reaction was terminated by the addition of 4.0 mL of alkaline hydroxylamine reagent and the residual concentration of acetylcholine was determined by Hestrin's colorimetric method. In each case, a plot of the percent inhibition of acetylcholinesterase vs. inhibitor concentration was used to determine the $I_{50}$ values. (Figure 2) The $I_{50}$ values reported represent the mean of at least six determinations.

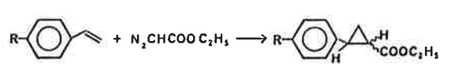
## RESULTS

The edge bonded cyclopropane analogs were prepared by the method outlined in *Scheme 1*. The *cis/trans* mixture of ethyl 2-phenylcyclopropane carboxylates, *14*, was generated by the addition of ethyl diazoacetate (EDA) to substituted styrenes. During the course of our work it became apparent from the work of Anciaux *et al.* (1980) that rhodium acetate could serve as a very effective catalyst for the generation of the carbenoid intermediate and make synthesis of these compounds more facile. We therefore undertook a study to determine the effect of rhodium acetate catalysis on the cyclopropanation of substituted styrenes. (See Table 1) The use of rhodium acetate allowed the reactions to occur at room temperature and increased the yields of cyclopropanation products even at catalyst concentrations as low as 0.10 mol percent and ratios of styrene to EDA which were significantly lower than those reported by Anciaux, *et al.* (1980).

Hydrolysis of the cyclopropanated esters and recrystallization of the resulting acids provided the pure trans isomers, *15*, which were then esterified with N,N-dimethylaminoethanol via an acid chloride intermediate. This procedure was used to generate the edge bonded compounds listed in Table 2 (6-9).

For the synthesis of the corner bonded analogs, 1-phenyl cyclopropanenitrile, *16*, was considered a key intermediate, since phenylacetonitrile could be cyclopropanated under a variety of conditions (*Scheme 2*) and the nitrile group could be hydrolyzed to the carboxylic acid *17*, which could then be esterified as before. Cyclopropanation of the phenylacetonitriles was first attempted using dibromoethane and 50% aqueous NaOH with a phase transfer catalyst following a procedure developed by Jonczyk, *et al.* (1971) for the cyclopropanation of phenylacetone. This method provided a low yield of pure material only after repeated fractional distillation and was considered impractical. We therefore turned to the method of Kaiser, *et al.*

TABLE 1. Cyclopropanation of Styrene with Ethyl Diazoacetate




| R | temp. °C | $Rh_2(OAc)_4$ Added | Styrene/EDA | Yield, %[a] |
|---|---|---|---|---|
| H | 135 | — | 10:1 | 55[b] |
| H | 25 | 1 mol % | 10:1 | 65 |
| H | 25 | 0.1 mol % | 10:1 | 86 |
| Cl | 135 | — | 10:1 | 50 |
| Cl | 25 | 0.33 mol % | 4.2:1 | 61[c] |
| Cl | 25 | 0.1 mol % | 10:1 | 60 |
| MeO | 135 | — | 10:1 | 10 |
| MeO | 25 | 0.33 mol % | 4:1 | 53 |
| MeO | 25 | 0.1 mol % | 1:1 | 42 |

[a] Isolated yield based on ethyl diazoacetate.

[b] *trans/cis* ratio 1.7 by $^1$H-NMR.

[c] *trans/cis* ratio 1.35 by $^1$H-NMR.

(1971) using alkylation of the dilithiated phenylacetonitrile dianion to provide the desired phenycyclopropane nitriles in good yield. The attempted acid catalyzed hydrolysis of the nitrile gave rise to a mixture of the desired 1-phenylcyclopropane carboxylic acid, *17*, and ring-opened 2-phenyl crotonic acid, but hydrolysis in aqueous base produced the desired acids, *17*, without accompanying ring opening. Esterification was accomplished as before. The corner bonded esters prepared by this procedure are listed in Table 2, (*10-13*).

For purposes of comparison, a number of substituted N,N-dialkylaminoethyl cinnamates were also prepared for evaluation of their local anesthetic potency, and these compounds are also listed in Table 2 (*1-5*).

All of the compounds thus prepared were screened for local anesthetic activity by the Goldfish overturn assay. The comparison among compounds was made using the turnover time measured for each compound at a standard defined concentration. (See Table 3) Several trends are apparent. The increasing activity in compounds *1-3* can be attributed to an increasing lipid solubility as the lipophilicity of the N-alkyl group increases. Substitution of either an electron withdrawing group (*4*) or an electron donating group (*5*) increased the activity relative to *1*. These same trends are observed in the edge bonded analogs, *6-9*, although the activity is slightly diminished relative to the standard olefinic set of compounds (*1-5*). The activity of the corner bonded analogs *10-13*, in general, is considerably less than the corresponding olefinic compounds. If, however, one compares the activity of the ring substituents in all three sets of compounds, it is apparent that the p-chloro group has a greater potentiating effect on the local anesthetic potency than does the p-methoxy group.

In an attempt to determine whether the activity of the cyclopropane compounds was due to a conjugative effect of the cyclopropane ring connecting the aromatic system with the carbonyl group, the ultraviolet spectral characteristics of the compounds were measured. The UV spectra of compounds *1, 6,* and *10* are shown in Figure 1. Compound *1* shows an intense absorption band at 281 nm ($\epsilon_{max}$ 24,900) which is characteristic of a K-band resulting from conjugation of the carbonyl group with the benzene ring. This is in direct contrast to the spectra of both types of cyclopropane derivatives which exhibit only weak absorption between 257 and 265 nm ($\epsilon_{max}$ 200-600) with the characteristic fine structure suggestive of a benzene chromophore.

A second bioassay was conducted based on the work of Rosenberg, *et al.* (1963) which showed that local anesthetics produced an inhibitory effect on purified acetylcholinesterase and were able to block the electrical activity of an isolated electroplax. It has recently been demonstrated that several tertiary amine local anesthetics indeed inhibit the enzymatic activity of acetylcholinesterase associated with rat brain synaptosomal membrane (Sidek *et al.* 1984) and $\psi$-cholinesterase from plasma and red blood cells (Alkondon *et al.* 1983).

The capability of compounds *1, 6* and *10* to inhibit the activity of acetylcholinesterase was therefore measured using a procedure adapted from Rosenburg *et al* (1963). (Table 3) The corner bonded analog, *10*, which was only about one-fourth as effective as *1* in the Goldfish assay proved to be about one-tenth as effective in the enzyme inhibition assay, whereas the edge bonded analog, *6*, which was nearly equipotent on Goldfish was about one-third as effective in inhibiting acetylcholinesterase. The $I_{50}$ values measured here compare quite favorably with those reported by Sidek *et al.* (1984) for synaptosomal acetylcholinesterase inhibition by procaine and lidocaine.

TABLE 3. Biological Effects of the N,N-Dialkylaminoethyl Cinnamates and Cyclopropyl Analogs.

| Compound | O T, sec[a] | R.A.[b] | $I_{50}$, mM[c] |
|---|---|---|---|
| *1* | 232 | 1.00 | 1.24 ± 0.40 |
| *2* | 92 | 2.52 | |
| *3* | 54 | 4.30 | |
| *4* | 153 | 1.52 | |
| *5* | 166 | 1.40 | |
| *6* | 247 | 0.94 | 3.55 ± 0.15 |
| *7* | 177 | 1.31 | |
| *8* | 84 | 2.76 | |
| *9* | 187 | 1.24 | |
| *10* | 954 | 0.24 | 11.7 ± 0.15 |
| *11* | 200 | 1.16 | |
| *12* | 942 | 0.25 | |
| *13* | 554 | 0.42 | |

[a] OT = Mean goldfish overturn time. All tests were conducted at 3 mM solution concentration of the test compound at 20°C.

[b] R.A. = Relative activity from the Goldfish assay for local anesthetic activity.

[c] $I_{50}$ = Concentration of compound necessary to provide 50% inhibition of the enzyme Acetylcholinesterase.

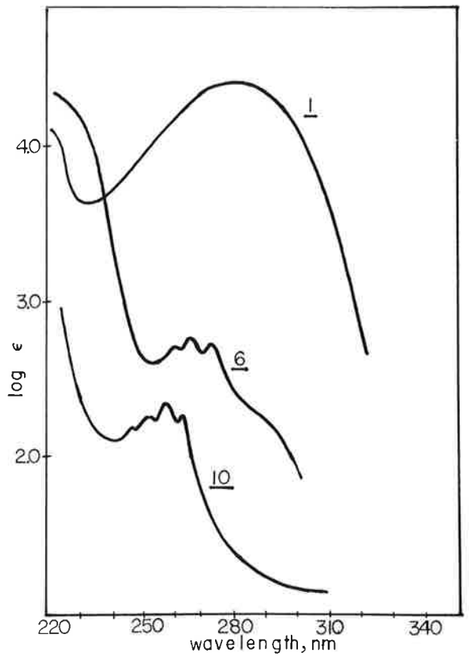



FIGURE 1. Ultraviolet Spectra of N,N-Dialkylaminoethyl Cinnamates and Cyclopropyl Analogs.

## DISCUSSION

A comparison of the ultraviolet spectra of the vinylic analog with the ultraviolet spectra of the two cyclopropane compounds leads one to conclude that, at best, conjugation is not as extensive in either cyclopropane compound as it is in the vinylic $\pi$-electron system. This result is not totally unexpected in view of the work of Wilcox *et al.* (1973) who stated that the same factor which makes cyclopropane an excellent stabilizing group by concentrating electron density at one site in its HOMO (thereby depleting other sites in the ring of electron density) assures that the group is not likely to be responsive to conjugative substituent effects.

Since all of the cyclopropane compounds demonstrated local anesthetic properties, this argues against the necessity of conjugation as a requisite for bioactivity, but it does verify the original hypothesis that the cyclopropane ring may be bioisosteric with the vinylic group in the local anesthetics synthesized in this study. If the bioactivity is not dependent on the conjugative effect, then perhaps the effect of the cyclopropane ring is purely steric in that it positions the proper molecular constituents in the appropriate locations to afford an optimum interaction with the bioreceptor.

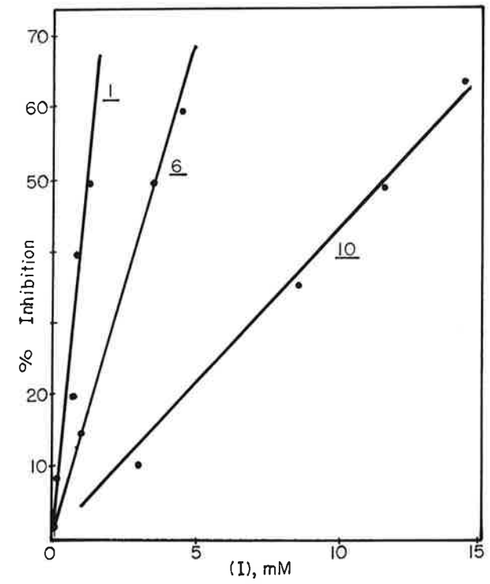



FIGURE 2. Inhibition of Acetylcholinesterase by N,N-Dialkylaminoethyl Cinnamates and Cyclopropyl Analogs.



## ACKNOWLEDGEMENT

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## LITERATURE CITED

Adams, R., E.K. Rideal, W.B. Burnett, R.L. Jenkins and E.E. Dreger. 1926. Chemical Constitution, Physiological Action and Physical Properties in a Series of Alkyl Para aminobenzoates. J. Am. Chem. Soc. 48:1758-1770.

Alkondon, M., A. Roy and P. Sen. 1983. Cholinesterase Enzyme Inhibition by Some Antiarrhythmic Drugs *in vitro*. Indian J. Pharmacol. 15:85-89.

Anciaux, A.J., A.J. Hubert, A.F. Noels, N. Petiniot and P. Teyssie. 1980. Transition-Metal-Catalyzed Reactions of Diazo Compounds. Cyclopropanation of Double Bonds. J. Org. Chem. 45:695-701.

Arai, Y., K. Shimoji, M. Konno, Y. Konishi, S. Okuyama, S. Iguchi, M. Hayashi, T. Miyamoto and M. Toda. 1983. Synthesis and 5-Lipoxygenase Inhibitory Activities of Eicosanoid Compounds. J. Med. Chem. 26:72-78.

Bernett, W.A. 1967. A Unified Theory of Bonding for Cyclopropanes. J. Chem. Ed. 44:17-24.

Blanchard, S.G., J. Elliott and M.A. Raftery. 1979. Interaction of Local Anesthetics with Torpedo Californica Membrane-Bound Acetylcholine Receptor. Biochemistry 18:5880-85.

Brill, H.C. and C.F. Cook. 1933. Some Alkamine Esters of Cinnamic Acid and Derivatives: Novocaine Analogs. J. Am. Chem. Soc. 55:2062-4.

Burger, A. and W.L. Yost. 1948. Arylcycloalkylamines I. 2-Phenylcyclopropylamine. J. Am. Chem. Soc. 70:2198-2201.

Burger, A., D.G. Markees, W.R. Nes and W.L. Yost. 1949. Analogs of Dibenzylacetic Acid. J. Am. Chem. Soc. 71:3307-10.

Chinn, L.J., K.W. Salamon and B.N. Desai. 1981. A Structure-Activity Relationship Study of Spirolactones. Contribution of the Cyclopropane Ring to Antimineralocorticoid Activity. J. Med. Chem. 24:1103-7.

Coulson, C.A. and W.E. Moffitt. 1949. The Properties of Certain Strained Hydrocarbons. Phil. Mag. 40:1-35.

Domagala, J.M., C.L. Heifetz, M.P. Hutt, T.F. Mich, J.B. Nichols, M. Solomon and D.F. Worth. 1988. 1-Substituted 7-[3-[(Ethylamino)methyl]-1-pyrrolidinyl-6, 8-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic Acids. New Quantitative Structure-Activity Relationships at N1 for the Quinolone Antibacterials. J. Med. Chem. 31:991-1001.

Feldman, S., M. De Francisco and P.J. Cascella. 1975. Activity of Local Anesthetic Agents in Goldfish. J. Pharm. Sci. 64:1713-15.

Galinsky, A.M., J.E. Gearien, A.J. Perkins and S.V. Susina. 1963. Electronic Effects of para-Substituents on the Local Anesthetic Activity of 2-Diethylaminoethyl Benzoate and Related Compounds. J. Med. Chem. 6:320-2.

Gerhards, J., T.K. Ha and X. Perlia. 1986. A PCILO Study of the Pharmacologically Active Conformation of Local Anesthetics of the Procaine Type. Arzneim. Forsch/ Drug Research 36(1):861-68.

Hestrin, S. 1949. The Reaction of Acetylcholine and Other Carboxylic Acid Derivatives with Hydroxylamine, and its Analytical Application. J. Biol. Chem. 180:249-261.

Hille, B. 1977. Local Anesthetics: Hydrophilic and Hydrophobic Pathways for the Drug-Receptor Reaction. J. Gen Physiol. 69:497-515.

House, H.O., W.C. McDaniel, R.F. Sieloff and D. Vanderveer. 1978. Perhydroindan Derivatives. Opening of a Cyclopropyl Ketone that is Part of an Indanone System. J. Org. Chem. 43:4316-23.

Jonczyk, A., B. Sarafin and M. Makosza. 1971. Reactions of Organic Anions. Catalytic Alkylation of Phenylacetone with $\alpha$, $\omega$-Dihaloalkanes in Aqueous Medium. Roczniki Chemii 45:2097-2105.

Kaiser, E.M., L.E. Salter, R.A. Schwarz, R.D. Beard and C.R. Hauser. 1971. gem-Dialkali Derivatives of Certain Functionally Substituted Organic Compounds. J. Am. Chem. Soc. 93:4237-42.

Knowles, E.C. and J.B. Cloke. 1932. Substituted Phenylacetonitriles and Derivatives. J. Am. Chem. Soc. 54:2028-37.

Loew, G.H. and D.S. Berkowitz. 1978. Quantum Chemical Studies of N-Substituent Variation in the Oxymorphine Series of Opiate Narcotics. J. Med. Chem. 21:101-6.

McNeal, E.T., G.A. Lewandowski, J.W. Daly and C.R. Creveling. 1985. [$^3$H] Batrachotoxinin A 20-$\alpha$-Benzoate Binding to Voltage-Sensitive Sodium Channels: A Rapid and Quantitative Assay for Local Anesthetic Activity in a Variety of Drugs. J. Med. Chem. 28:381-88.

Neher, E. and J.H. Steinbach. 1978. Local Anesthetics Transiently Block Currents Through Single Acetylcholine-Receptor Channels. J. Physiol. 277:153-176.

Pews, R.G. and N.D. Ojha. 1969. The Electronic Properties of Three-Membered Rings. The Transmission of Conjugation by the Cyclopropane Ring. J. Am. Chem. Soc. 91:5769-73.

Rosenberg, P. H.B. Higman and E. Bartels. 1963. The Active Structure of Local Anesthetics. Effects on Electrical and Cholinesterase Activity. Biochim. Biophys. Acta 66:406-14.

Sidek, H.M., C. Nyquist-Battie and G. Vanderkooi. 1984. Inhibition of Synaptosomal Enzymes by Local Anesthetics. Biochim. Biophys. Acta. 801:26-31.

Weston, A. 1946. Antispasmodics. Derivatives of 1-Phenylcycloparaffin Carboxylic Acids. J. Am. Chem. Soc. 68:2345-48.

Wilcox, C.F., L.M. Loew and R. Hoffman. 1973. Why a Cyclopropyl Group Is Good at Stabilizing a Cation but Poor at Transmitting Substituent Effects. J. Am. Chem. Soc. 95:8192-3.

Witjens, P.H. C.P. Van Dijk and P.E. Verkade. 1947. Investigation of the Usefulness of the Goldfish Test for the Determination of Local Anesthetic Activity. Archiv. Int. Pharmacodyn. 74:378-398.

APPENDIX.
Elemental Analyses of N,N-Dialkylaminoethyl Cinnamates and Cyclopropyl Analogs.

| Compound Number | %, Calculated C | %, Calculated H | %, Found C | %, Found H |
|---|---|---|---|---|
| *1* | 61.05 | 7.09 | 60.78 | 7.00 |
| *2* | 63.48 | 7.81 | 63.55 | 7.73 |
| *3* | 65.47 | 8.40 | 65.25 | 8.45 |
| *4* | 53.80 | 5.91 | 53.73 | 5.94 |
| *5* | 58.84 | 7.05 | 58.64 | 7.18 |
| *6* | 62.33 | 7.47 | 62.10 | 7.54 |
| *7* | 64.52 | 8.12 | 64.38 | 8.15 |
| *8* | 55.27 | 6.29 | 54.93 | 6.63 |
| *9* | 60.09 | 7.40 | 60.46 | 7.59 |
| *10* | 62.33 | 7.47 | 62.18 | 7.58 |
| *11* | 55.27 | 6.29 | 55.32 | 6.29 |
| *12* | 60.09 | 7.40 | 59.80 | 7.52 |
| *13* | 62.28 | 7.99 | 62.68 | 7.99 |

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# DIRECTIONAL PROFILES AND ROSE DIAGRAMS TO COMPLEMENT CENTROGRAPHIC CARTOGRAPHY[1]

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## ABSTRACT

**By collapsing magnitude data for many points or areal units to a single weighted mean center, the centrographic map promotes the concise spatial-temporal analysis of individual geographic distributions as well as the visual examination of generalized relationships among two or more geographic distributions. Although a more thorough analysis calls for cartographic display of the dispersion of data values about each mean center, standard-distance symbols, dispersion ellipses, and other existing methods for representing dispersion do not adequately portray the asymmetry of most geographic patterns. This paper develops and demonstrates two new approaches to portraying dispersion, the azimuth-frequency rose diagram and the cardinal-direction profile. In addition to providing a more informative centrographic analysis, these new graphic devices are also useful for animated statistical maps and for the interactive cartographic analysis of geographic correlation.**
**[J PA Acad Sci 66(1):29-34, 1992]**

## INTRODUCTION

This paper addresses a geographic technique called *centrography*, which uses a single point to represent center of mass for a geographic distribution such as population, value added in manufacturing, or number of automobiles. As Figure 1 illustrates, centrographic analysis can examine movement over time of a single geographic distribution's national or local center, or compare geographic centers for two or more related distributions.

Julius Hilgard (1872) described the first centrographic analysis in 1872. A cartographer for the U.S. Coast Survey, Hilgard estimated demographic centers by computing arithmetic means for latitude and longitude weighted by population.[1] His mass points consisted of cities with more than 50,000 inhabitants, and for the balance of the population, the center of area for each state. In 1874, the *Statistical Atlas of the United States* (Walker and others, 1874) used the first published centrographic time-series map to describe the westward movement of the United States population decade-by-decade between 1790 and 1870. During the 1920s and 1930s, early quantitative geographers in Russia and the United States employed "centrography" to study a variety of geographic patterns (Sviatlovsky and Eells, 1937). Centrographic analysis attracted notable attention during the social physics movement of the 1950s, the "quantitative revolution in geography" of the early 1960s, and the development of quantitative social-behavioral geography in the late 1970s (Kellerman, 1981, 25). Recent developments in animated cartography and interactive computer graphics suggest that the centrographic map has important roles in dynamic cartography as both a summary graphic and a tool for the exploratory cartographic analysis of spatial-temporal data (Monmonier, 1990 and 1992).

This paper is concerned with representing the dispersion of mass around a geographic distribution's mean center. Common measures of dispersion in centrographic analysis are the *standard distance* and the *cross of dispersion* (Kellerman, 1981, 12-25). Figure 2 illustrates the cartographic representation of these two measures for eight simple configurations of mass points: a circle centered on the mean center portrays standard distance, and an ellipse shows the cross of dispersion.[2] Although the dispersion ellipse better represents the spatial pattern of mass units than the standard distance or any other single measure of dispersion, its two axes of symmetry preclude showing the essential characteristics of the less symmetric patterns in Figure 2. Centrographic analysis and dynamic cartography require a more informative, more readily interpreted portrayal of dispersion about thematic geographic centers.

## MATERIALS AND METHODS

The two approaches proposed here are cardinal-direction profiles and the azimuth rose. The former provides separate graphic summaries for the north-south and west-east directions, which most viewers should find meaningful, whereas the latter uses an eight-point compass rose to describe the azimuthal distribution of mass about the mean center.

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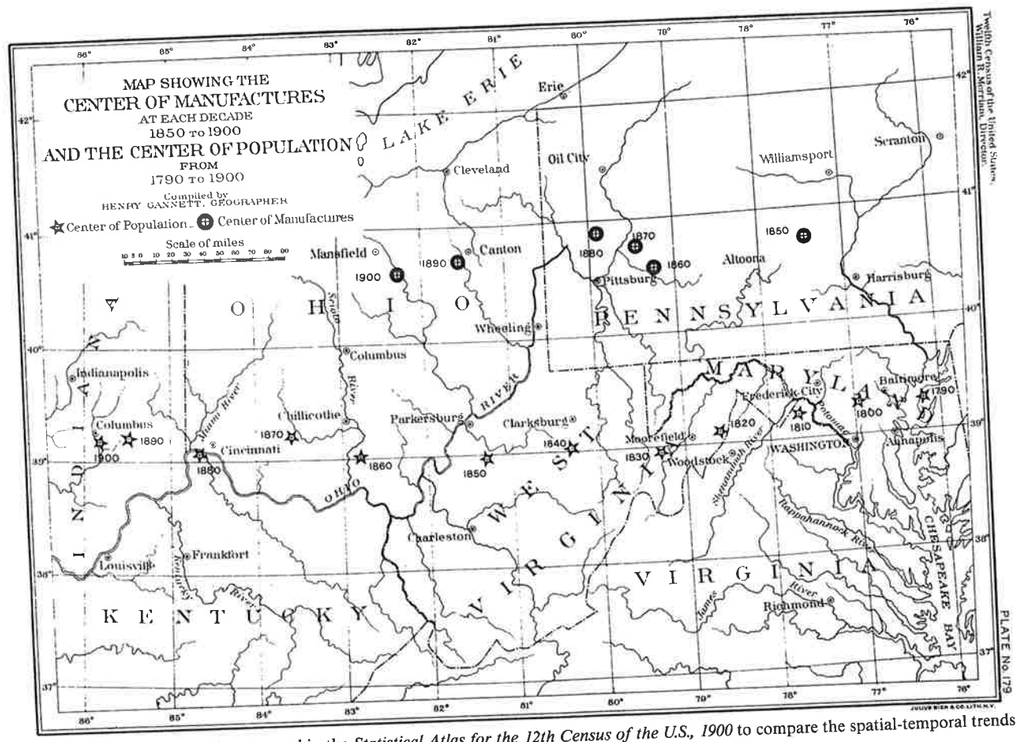


FIGURE 1. Centrographic map used in the *Statistical Atlas for the 12th Census of the U.S., 1900* to compare the spatial-temporal trends of population and value of manufactured goods (U.S. Census Office, 1903, plate 179).

### *Cardinal-Direction Profiles*

Figure 3 employs a simple five-area region to describe the conceptual basis of the cardinal-direction profile. Although the diagram describes calculations only for the longitudinal, or west-east, profile, a similar process produces the latitudinal, or north-south, profile. The process assumes magnitude data or counts for each area, rather than intensity data, such as percentage rates, medians, or rates of change.

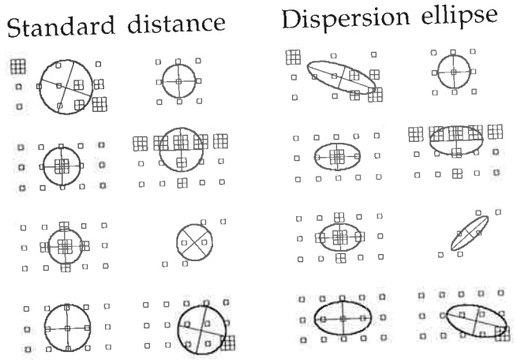


FIGURE 2. Circles and ellipses representing the standard distance and the cross of dispersion which can hide salient patterns in the asymmetric distribution of mass around the mean center. Radius of the circle is proportional to half the standard distance. Major and minor axes of the ellipse are the maximum and minimum directional variances.

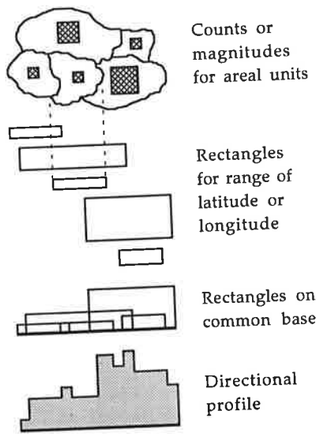


FIGURE 3. Stages in the development of a longitudinal frequency profile (bottom) for a hypothetical region with five areal units (top).



Calculations begin by finding the minimum and maximum longitudes $\lambda_{min.i}$ and $\lambda_{max.i}$ for each areal unit.[3] These extremes are used to generate a rectangle that distributes the count for the area uniformly over the range of longitude. For area i the height $h_i$ of this rectangle is computed by dividing the magnitude or weight $W_i$ by the range of longitude for the data area, according to

$$h_i = W_i / (\lambda_{max.i} - \lambda_{min.i}).$$

This calculation generates a pair of two-part records for each area: $(\lambda_{min.i}, +h_i)$ and $(\lambda_{max.i}, -h_i)$. The algorithm accumulates these records in a list, which is sorted according to the longitude coordinate $\lambda$. The plot for a longitudinal frequency profile starts at the left, with the lowest longitude in the list, and proceeds to the right. For each successive record in the list, the sign for the height of the associated rectangle specifies whether the profile is raised or lowered.[4]

For point data a cumulative profile could replace the frequency profile, although this author's experience suggests that viewers cannot as readily interpret or compare graphs of cumulative distributions. A frequency profile similar to that described for area data in Figure 3 can be based on rectangles or triangles if each point location is treated as a standard circular area.[5] The analyst should explore the effects of variations in circle size on the shape of the cardinal-direction profile as well as have a rational basis for setting this zone of influence. For data on retail sales, for instance, the radius of the circle might reflect how far an average shopper is willing to travel.

Standardizing the area under the profile promotes comparison of cardinal-direction profiles plotted for different times or different phenomena. A square or series of squares representing 1, 5, or 10 percent of the area under the profile might prove a useful key. Useful too is the addition to the profile diagram of labeled markers or vertical lines representing (a) the mean weighted latitude or longitude and (b) the median position that divides the area under the profile into two equal portions. The median position can be estimated easily from the list used to generate the profile.[6]

### *The Azimuth Rose*

The azimuth rose is a simple frequency diagram that describes the relative distribution of mass among eight 45-degree-wide sectors centered on the geographic distribution's arithmetic mean center. A graduated bar represents the direction and frequency for each sector. Each sector is centered on one of the eight directions of a basic compass card that includes the four cardinal directions and their four intermediate divisions: northeast, southeast, southwest, and northwest. Because these directions should be familiar to most viewers, labels are not required.

Developing an azimuth rose begins by defining an oblique azimuthal projection tangent to the globe at the computed mean center and calculating the azimuth of each data point in degrees clockwise from north (Snyder, 1987). These azimuths are used to assign the weight $W_i$ for each data point to one of eight frequency bins. Radiating bars or lines representing relative frequency are plotted for each directional sector.

Figure 4 shows an azimuth rose for eight sample mass configurations with varying degrees of asymmetry. These examples demonstrate that the azimuth rose can be an effective graphic device for describing an asymmetric pattern of geographic mass around the mean center. Consider, for instance, the example in the upper right, where a three-pronged rose diagram describes the slightly eccentric mean center chiefly as the result of large masses to the north, the southwest and the southeast. That the diagram treats the large mass point in the first row and first column of this configuration as north, not northwest, reflects the generalization of azimuths to only eight principal directions aligned with north.

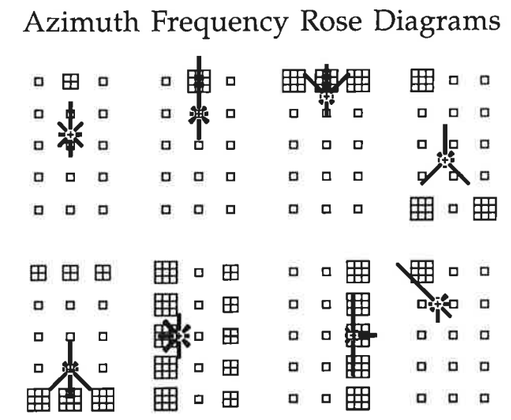


FIGURE 4. Azimuth roses reflect varying degrees of asymmetry among eight mass configurations.

## RESULTS AND DISCUSSION

Demonstration graphics based on state-level newspaper-circulation and adult-population data were used to test and explore the utility of cardinal-direction profiles and azimuth roses.[7] Figure 5 shows mean centers for both distributions at a five-year interval over the period 1960 to 1985[8]. The time-track lines linking successive mean centers illustrate the generally progressive shift of both distributions toward the southwest. That the center of newspaper circulation is consistently east and north of the geographic center of the adult population reflects generally higher rates of newspaper readership among the somewhat older population in the northeast.[9]

Figure 5 also reveals a serious deficiency of newspaper-circulation data tabulated by corporate address. The eastward reversal of the newspaper-circulation time track between 1980 and 1985 most likely reflects the rise of large-

circulation national newspapers such as *USA Today* and the *Wall Street Journal*, distributed from various printing sites throughout the country but reported in the respective circulation tallies for the District of Columbia and New York State.

Azimuth roses for 1960 and 1985 reflect greater symmetry in the north-south direction than in the west-east direction. Yet differences among the four azimuth roses in Figure 5 are slight. Although possibly useful at the beginning and end of time-track lines, the rose diagram seems not to be particularly informative for illustrating gradual, evolutionary change or for comparing subtly different distributional patterns. But for more radically different patterns, particularly with widely separated mean centers, the azimuth rose might be a usefully revealing cartographic symbol.

In contrast, the cardinal-direction profile can be highly informative even when change is slight. Figure 6, which compares the patterns of newspaper circulation for 1960 and 1985, supplements the centrographic map by revealing a valley of low circulation between the Mississippi and the Pacific Coast. Comparison of the two profiles indicates noticeable relative growth along the country's southern and western margins.[10] The north-south profiles in this example reflect relative circulation growth not only in "sunbelt" states but at a middle-latitude peak roughly coincident with Philadelphia, Baltimore-Washington, Cincinnati, St. Louis, Kansas City, Denver, and San Francisco.

Vertical lines representing the directional median and triangular markers showing the mean center enhance the informativeness of directional profiles. Juxtaposition, with corresponding diagrams for different years stacked vertically, as in Figure 6, helps point out temporal shifts in both mean and median as well as promotes an examination of regional trends contributing to national shifts.

Figure 7, which compares newspaper circulation with adult population for 1985, shows an even more pronounced mid-latitude peak for population, reflecting lower circulation rates. Indeed, superimposing these profiles would indicate a general decline from north to south in per capita newspaper circulation.

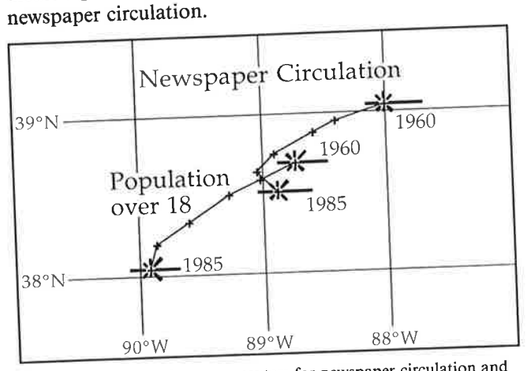


FIGURE 5. Geographic mean centers for newspaper circulation and adult population, 1960-1985. Time tracks show movement of the geographic center at a five-year interval, and azimuth rose shows general distributuon around the mean center for 1960 and 1985.

Individual profiles in Figures 6 and 7 are scaled vertically to an identical maximum height. This equal-height strategy would point out shifts in the modal area as well as provide maximum geographic detail within individual profiles. Other vertical-scaling strategies might promote visual comparison by standardizing area under the curve for a series of profiles or by using a constant vertical scale for a phenomenon viewed at different times.

## CONCLUSION

These examples indicate that the directional profile and, to a lesser extent, the azimuth rose can be valuable complements to the centrographic map. By providing a more complete graphic description of dispersion about the mean center, both new symbols clearly are more informative than either the standard-distance circle or the dispersion ellipse. One particularly promising example of this complementarity would be an animated sequence in which a time track on a map illustrates the cumulative movement of the geographic center while directional profiles illustrate the generalized north-south and west-east patterns for individual instants or periods of time.

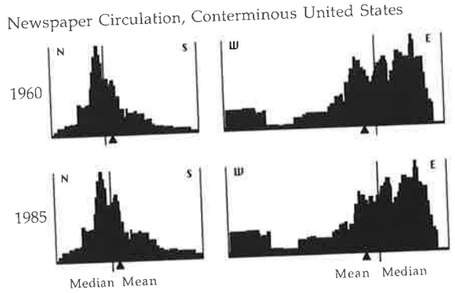


FIGURE 6. Cardinal-direction profiles comparing the national attern of newspaper circulation for 1960 and 1985.

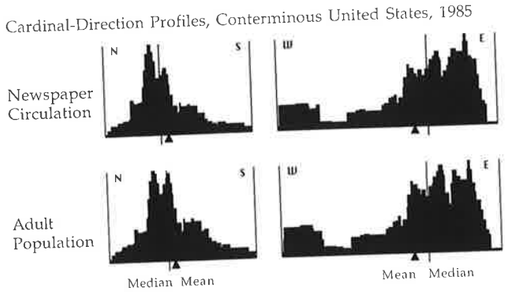


FIGURE 7. Cardinal-direction profiles comparing the national patterns for 1985 of newspaper circulation and the adult population.



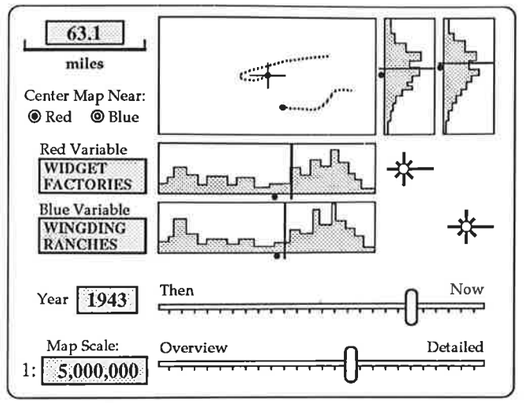


FIGURE 8. Organization of key elements on the display screen of a hypothetical visualization support system for analyzing spatial-temporal patterns of bivariate geographic correlation. The analyst would use the radio buttons to center the screen near either the red or the blue variable's time track, and the slider bars to select the year and the scale of the map.

Directional profiles and azimuth roses can be valuable supplementary graphics in experiential cartography, particularly for the interactive exploration of geographic correlation. Figure 8 illustrates a hypothetical display screen on which the colors warm red and dark blue represent two different variables. These colors link each variable's name to its time track, directional profile, and azimuth rose. A direct-manipulation interface provides "slider bars" for selecting the year and the scale of the map as well as "radio buttons" for centering the map window near either the "red" or the "blue" time track. By manipulating time and scale for this and a variety of other cartographic representations, the analyst might gain a fuller understanding of meaningful relationships among the measurements in a spatial-temporal data set.

## ACKNOWLEDGEMENT

The support of the National Science Foundation (SES-90-22845) is gratefully acknowledged.

## ENDNOTES

1. The U.S. Bureau of the Census (1983) adjusts for the sphericity of the earth by computing the weighted mean center as

   $$\phi_c = \Sigma W_i\phi_i \,/\, \Sigma W_i$$

   for latitude, and as

   $$\lambda_c = \Sigma W_i\lambda_i \cos\phi_i \,/\, \Sigma W_i \cos\phi_i$$

   for longitude, where $\phi_i$, $\lambda_i$, and $W_i$ are, respectively, the latitude, longitude, and weight (say, population) of point i.

2. The standard distance is based on the standard deviation and computed as the weighted average distance around the mean center, according to

   $$d_s = [\,(\Sigma W_i(\phi_i - \phi_c)^2 + \Sigma W_i((\cos\phi_i + \cos\phi_c)/2)(\lambda_i - \lambda_c)^2)\,/\,(\Sigma W_i)\,]^{0.5}.$$

   The cross of dispersion is based on the maximum and minimum dispersion components $\sigma_{max}^2$ and $\sigma_{min}^2$, which are measured on orthogonal axes through the mean center and define the maximum and minimum dispersion around the mean center (Lefever, 1926). They serve as major and minor axes of the ellipse of dispersion and are related to standard distance according to

   $$d_s^2 = \sigma_{max}^2 + \sigma_{min}^2.$$

   For further discussion, see Kellerman (1981, 16-24) and Neft (1966, 55-58). To promote comparison, radii of circles in Figure 2 are proportional to half the standard distance.
3. For relatively small regions such as individual states or metropolitan areas, the plane coordinates (northing and easting) may be substituted for spherical coordinates (latitude and longitude). In this case, centers should be plotted on a conformal map related to the plane-coordinate system.
4. When one area ends at the latitude or longitude where another starts, additional sorting and smoothing is needed to avoid spurious 'spikes' in the profile.
5. *Binning*, the accumulation of measured amounts in a lattice of *bins* or grid cells, could be a useful strategy for developing cardinal-direction profiles for massive sets of point data. In this approach, magnitudes in the cells of a rectangular tessilation based on meridians and parallels could be summed in both directions to generate frequency counts for an orthogonal pair of frequency histograms or distribution-density plots. For a comprehensive discussion of binning, see Carr (1991).
6. The total area under the profile is the sum of the areas of all of the rectangles. The median can be estimated by maintaining a running tally of cumulative area while generating the profile from left to right and using linear interpolation to estimate the median position between the pair of successive profile points just under and just over 50 percent of the total area. Although a median center defined by the latitudinal and longitudinal medians can be plotted on the centrographic map, this point can be misleading because, unlike the mean center, a median center defined in this manner is not invariant under rotation (Neft, 1966, 29-33).
7. Newspaper circulation data are from an annual compilation published in the *Editor and Publisher Yearbook*. Population data are from the *Statistical Abstract of the United States*; values for 1985 are

intercensal estimates calculated by the Bureau of the Census, whereas values for 1965 and 1975 are averages interpolated between 1960 and 1970 and between 1970 and 1980, respectively. Centrographic calculations omitted data for Alaska and Hawaii, and combined data for the District of Columbia with those for Maryland. Reported by state of publication, these newspaper circulation data overestimate readership in states publishing national newspapers and other newspapers circulated heavily beyond a single state's borders.

8. Because they are based on state-level data and omit persons younger than 18, the population centers in Figure 5 differ slightly from the population centers on centrographic maps published in the national summary reports for the Census of Population and the annual *Statistical Abstract*. The Bureau of the Census uses county-unit data for its centrographic calculations.
9. For discussion of demographic trends in newspaper readership, see Benjaminson (1984) and Smith (1980).
10. Most of this "growth" is relative because between 1960 and 1985 total average daily newspaper circulation increased only slightly, from 59 to 63 million.

## LITERATURE CITED


Bachi, R. 1957. Statistical analysis of geographical series. Bulletin de l'Institute International de Statistique 36:229-240.

Benjaminson, P. 1984. Death in the Afternoon. Andrews, McMeel and Parker, Kansas City, KS.

Carr, D.B. 1991. Looking at large data sets using binned data plots. Forthcoming in Turkey, P. and A. Buja, eds. Computational Statistics and Graphics. Springer Verlag, New York.

Hilgard, J.E. 1872. The advance of population in the United States. Scribner's Monthly 4(2):214-218.

Kellerman, A. 1981. Centrographic Measures in Geography. Concepts and Methods in Modern Geography monograph no. 32. Geo Abstracts, Univ. of East Anglia, Norwich, UK.

Lefever, D.W. 1926. Measuring geographic concentration by means of the standard deviational ellipse. Am. J. Sociology 32:88-94.

Monmonier, M. 1990. Strategies for the visualization of geographic time-series data. Cartographica 27(1):30-45.

_____. 1992. Summary graphics for integrated visualization in dynamic cartography. Cartography and Geographic Information Systems 19: forthcoming.

Neft, D.S. 1966. Statistical Analysis for Areal Distributions. RSRI monograph no. 2. Regional Science Research Institute, Philadelphia.

Smith, A. 1980. Goodbye Guttenberg: the Newspaper Revolution of the 1980s. Oxford University Press, New York.

Snyder, J.P. 1987. Map Projections—A Working Manual. USGS professional paper no. 1395. U.S. Geological Survey, Reston, VA, 30.

Sviatlovsky, E.E. and W.C. Eells. 1937. The centrographic method and regional analysis. Geographical Review 27:240-254.

U.S. Bureau of the Census. 1974. Centers of Population for States and Counties. Government document no. C56.202:P81/3.

_____. 1983. Census of Population: 1980. Characteristics of the Population. Number of Inhabitants. United States Summary. Report PC80-1-A1. p. A-5.

U.S. Census Office. 1903. Statistical Atlas for the 12th Census, 1900. Washington, DC.

Walker, F.A. and others. 1874. Statistical Atlas of the United States. U.S. Census Office, Washington, DC, 6.


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# A CONCEPT-COORDINATED ESCAPE REFLEX IN THE MONGOLIAN GERBIL[1]

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## ABSTRACT

**Following Skinner (1935), reflexive escape behavior was defined as a coordination between a response class and a stimulus class. Since induction is an implied characteristic of reflexes, the phenomenon of induction was sought for gerbils' gnaw-to-escape behavior. It was found that for the condition in which the ratio of the volumes of the start chamber to the escape chamber was 1 to 4, the average escape latency for a group of gerbils was shorter if they had had previous experience in a condition in which the ratio of the start chamber volume to escape chamber volume was 1 to 1. The converse was also found in that a group's average escape latency was longer in a second 1 to 1 chamber condition if a previous condition of 1 to 4 had been experienced. These results provided support for the depiction of escape as a reflex with broadly defined stimulus and response classes.**
**[J PA Acad Sci 66(1):35-38, 1992]**


## INTRODUCTION

A concept is an organism's representation of the relationships between its life goals, behavior and environmental events or conditions. One means of demonstrating a concept is through the type of reflex described by Dewey (1896). Dewey argued that the prevalent view of the reflex arc unsuitably assumed that a stimulus could be understood just by its physical characteristics (and the immediate sensations that they produced), quite apart from the coordination of the stimulus with its associated reflexive response. He warned that a reflex should not be separated into units of sensation, central processing, and motor response, but instead should be considered as a coordination among these entities, whose function is to achieve an organism-determined goal. The type of reflex that Dewey described is a good description of conceptual behavior and, hence, one may conclude that the identification of a reflex and the discovery of a concept is a similar enterprise.

A reflex is defined as "a correlation of a stimulus and a response at a level of restriction marked by the orderliness of changes in the correlation" (Skinner, 1935, p. 55). To begin the process of reflex identification, consider a Mongolian gerbil (*Meriones unguiculatus*) when first introduced to a field of loose substrate (i.e., sawdust, sand, wood chips, etc.) The subject will actively move about the field and very little digging behavior will be emitted within the first few moments of introduction. However, if confined upon the field, the gerbil will dig within a short time at the boundary of its confinement and, if the physical structure of its confinement allows, it will dig through the substrate and pass through the boundary. When returned to the same or similar confining situation, it will dig at the boundary at the location of its previous successful experience. The gerbil may be returned repeatedly and will repeatedly exhibit the behavior as described (Kotary, Cleary & Drews, 1990).

One way to define the properties of the response class of this dig-to-escape reflex would be to categorize the responses that comprise a successful escape. Gerbil digging movements are part of the escape and they may be put into four categories:

1. clasping the substrate with both forepaws and pulling it underneath the body;
2. the same as category one except that the forepaws alternate;
3. moving the substrate from underneath the body by clasping it with both hindpaws and kicking it away; and
4. pushing substrate with the snout in a bulldozing fashion.

A continued expression of these movements results in a depression in the loose substrate at the location where it is performed.

Another feature of digging behavior is that it is directional, being performed at some point at the confining barrier. Hence, the animal's body location and orientation become part of the successful response pattern. A combination of the two behaviors, movement to a proper location with proper body orientation and digging motions with the

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limbs is one way to define the response class of the dig-to-escape reflex.

Measures of this response class may be either categorical or operational. A categorical measure would record the rate, location and direction of all of the categories of digging movements. An operational measure would rely upon the effect of digging movements and would record the time from confinement to the point at which the subject is through the barrier (termed escape latency). Either one of these measures will indicate the subject's level of escape behavior as long as the environmental context is one that elicits escape in the form of directed digging. There is an advantage, however, to using a latency measure in that it represents the subject's escape behavior in one number.

The definition of the stimulus class of the dig-to-escape reflex might begin with a minimal, physical description of the confinement stimulus. The confinement stimulus is a barrier which completely surrounds the subject, a barrier being a surface that obstructs movement. In order to evoke digging movements, some part of the barrier must be loose, digable material. It is the association of digging movements with the presentation of a confinement stimulus that is the central correlation of the dig-to-escape reflex.

The escape response discussed so far has been digging, but other escape-related behaviors are performed by a gerbil when surrounded by a barrier. To restrict the escape reflex to a digging response may be too narrow of a restriction. Some other escape-related activities include gnawing at the barrier, jumping, and climbing. When the environmental context allows these activities to be successful, they become the responses evoked by the confining stimulus. The first introduction to a confinement stimulus can lead to the evocation of a combination of these strategies, but successful experience focuses upon one, with the result that a correlation between a stimulus and a relatively limited response is created. Since it is the environmental configuration that determines which strategy is allowed or disallowed, it can be said that the environment selects the escape response. Therefore, one of the defining features of the confinement stimulus class is its ability to select a particular strategy of escape and this ability comes through the interpretations of the goal-directed organism.

In a situation similar to the selection of escape behavior just described for the Mongolian gerbil, Blanchard, Blanchard & Hori (1989) showed that environmental context can select certain types of fear-based defensive behaviors in the laboratory rat (*Rattus norvegicus*). In contact with a predator, the rat would choose a flight or would choose a freezing defensive response, depending upon the flight possibilities of the situation. In addition, they showed that environmental context can also select different types of anxiety-based risk assessment behaviors, depending upon the magnitude of the threat and the type of information that the threat makes available.

Selection of escape behavior is a characteristic of the stimulus class of an escape reflex. Since the perception of the stimulus class, like other perceptions, may be achieved through variable means (Koffka, 1935), it is difficult to find the defining properties of the escape reflex stimulus in *just* the physical features of the stimulus configuration. Dewey (1896) foresaw this difficulty.

The level of restriction of the stimulus class needs to include more than a physical description of a surrounding barrier. At a higher level of restriction, the response class would be a set of behaviors that operate to overcome the boundaries of confinement and the stimulus class would be a set of environmental configurations that have in common the characteristics of 1. surrounding a subject with perceived barriers and 2. being able to select a particular escape strategy. Even though these class definitions include a variety of response types and stimulus possibilities, a central correlation nevertheless holds between them and a reflex is indicated.

A second step in identifying a reflex is to search for the operation of secondary laws, specifically the process of induction during repeated elicitation of the reflex. Skinner (1935) defined induction as "a process through which a change in the state of one reflex is said to induce a similar change in the state of another" (p. 47). Since the escape reflex involves a change in the state of the perceived confining stimulus, variations in the degree of this change should produce orderly variations in the escape response. Induction would be demonstrated if the effects of repeated experiences under one degree of confinement change carry over to, or induce, the escape response found under a second degree of confinement change. The induction effect would be found because these stimulus configurations are presented in sequence and because both are within the stimulus class of the escape reflex.

If the broader definition of an escape reflex presented so far is to hold, the phenomenon of induction during secondary changes should be found for the types of behaviors classified as escape. The experiment to be reported addresses the question of induction in gnaw-to-escape behavior.

## MATERIALS AND METHODS

Two litters of six Mongolian gerbils *(Meriones unguiculatus)* each were used in the experiment. The litters were from two unrelated sets of parents. One litter was 14 months old at the time of the experiment and the other litter was 10 months old at the time of the experiment. Of the 14 month old litter, subjects 22M, 23M, 24M and 27M were male, subjects 29F and 30F were female. Of the 10 month old litter, subjects 40M, 41M and 42M were male, subjects 43F, 45F and 46F were female.

All subjects were experimentally naive. Each of the litters were separated from their parents at approximately 3 months of age and were housed with littermates in a glass terrarium with free access to food, water and a running wheel.

The apparatus consisted of two attached hardware cloth rectangular chambers, enclosed on all sides and the top, with the bottom open. A 5 x 5 cm opening allowed access from one chamber to the other. The opening was covered by a 5.5 x 10 cm section of white cardboard. The weight of the cardboard was 0.0345 g/cm$^2$. The cardboard had a 2 cm diameter hole punched in its center and this circular hole was aligned so that it was in the center of the square opening between the two hardware cloth chambers. The chambers were anchored by two 1 kg weights.

There were two chamber sizes, the smaller size was 13 x 13 x 13 cm and the larger size was four times the volume of the smaller, its dimensions being 26 x 26 x 13 cm.

The gerbil's escape latency was the dependent measure. Escape consisted of gnawing the edges of the hole in the cardboard barrier between the two chambers until an enlarged hole was created and the gerbil passed through it.

A subject was placed into the escape apparatus by tilting the chamber up and placing the subject on the surface that would be covered by the starting chamber, then lowering the chamber over the subject. A subject was always placed facing away from the escape opening. A timer was initiated immediately after this was done and the timer was stopped when the subject had passed more than half of its body through the cardboard barrier.

For each group and every condition, the starting chamber was 13 x 13 x 13 cm in size and always oriented so that the other chamber was to the north.

The experiment was conducted on a 1.22 x 1.52 m table in the center of a 4.57 x 5.18 m windowless laboratory room, illuminated by fluorescent tube lighting. The observer, after placing the subject in the apparatus, would sit and make observations from a desk located 1.83 m from the escape apparatus table.

After the completion of an escape, the subject remained in the chamber apparatus for one minute, then was removed and placed in a 20 x 24 x 20 cm stainless steel cage. The gnawed cardboard barrier was replaced with a new one and the table surface was swept. The procedure was repeated two more times so that the three escape trials were performed per day. The first stage of the experiment was conducted for five consecutive days and was conducted approximately between the hours of 9 a.m. to 11 a.m.

The second stage of the experiment was conducted during the next five consecutive days during the same hours and continued the procedure just described. The ratio of the starting chamber size to the other chamber size was changed, however. During the first experimental stage, for group 1 subjects 22M, 23M, 29F, 40M, 41M and 46F, the chamber into which the subject escaped was the same size as the starting chamber. During the second stage, however, the chamber escaped into was changed to four times the volume of the starting chamber. The reverse was true for group 2 subjects 24M, 27M, 30F, 42M, 43F and 45F; the order of presentation of the chamber volume ratios was one to four in the first experimental stage and then was one to one in the second stage. Random assignment determined group membership.



## RESULTS

FIGURE 1 shows average escape latencies for the two groups under each of the two presentation orders. For descriptive purposes, the group that began in stage one with a chamber ratio of one to one and then was switched in stage two to a chamber ratio of one to four will be termed Group 1:1 to 1:4. The group with the reverse order of presentation will be termed Group 1:4 to 1:1. The escape times are listed according to the two types of chamber volume ratios in the order of their presentation. In the condition in which the chamber volume ratio was 1 to 1, the average escape latencies were longer for Group 1:1 to 1:4 as compared to Group 1:4 to 1:1. In the condition in which the chamber volume ratio was 1 to 4, the average escape latencies were also longer for Group 1:1 to 1:4 as compared to Group 1:4 to 1:1.

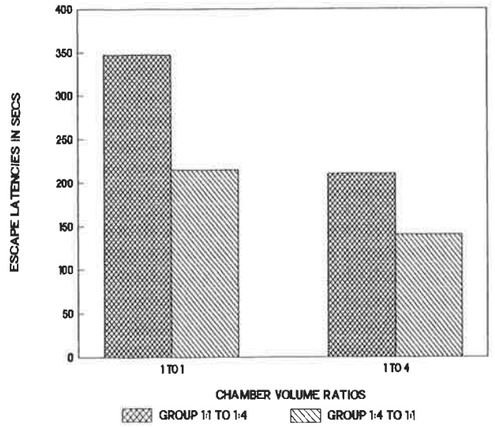


FIGURE 1. Average escape latencies under two chamber volume ratios and two presentation orders.

A two-factor mixed analysis of variance (ANOVA) indicated that these differences were statistically significant. The mixed ANOVA was for independent group measures on the order of presentation and for repeated measures on the chamber volume ratios. The analysis showed a significant main effect for presentation order, $F(1, 10) = 5.23$, $p = 0.045$, and showed a significant main effect for chamber volume ratio, $F(1, 10) = 21.91$, $p = 0.001$. The interaction was not significant, $F(1, 10) = 1.85$.

## DISCUSSION

When the start chamber to escape chamber volume ratio was one to four, repeated escape elicitation yielded comparatively short escape latencies. When the chamber volume ratio was one to one, comparatively long escape latencies were observed. Switching a group of gerbils from

the short escape latency condition to the long latency condition (Group 1:4 to 1:1) resulted in an increase in their escape times, but not to the level of the group that originally started in that condition (Group 1:1 to 1:4). Experience in the shorter escape latency condition may be said to have induced shorter escape times.

Conversely, switching a group of gerbils from the long escape latency condition to the short latency condition (Group 1:1 to 1:4) resulted in a decrease in their escape times, but not to the level of the group that originally started in that condition (Group 1:4 to 1:1). Experience in the longer escape latency condition may be said to have induced longer escape times.

According to Skinner (1935), the properties of the stimulus and response classes that make successive elicitation more similar to each other can identify a reflex. The induction of performance level from one type of reflex condition to another, just described in the present study, supports the view that escape behavior may reasonably be considered a reflex.

An escape reflex is defined by the coordination between its response class and its stimulus class. Whether or not it is possible for a stimulus to be defined independently of correlated behavior has historically been an area of disagreement in psychology (Gibson, 1960). An assumption of this study has been that it is misguided to use a stimulus to explain reflexive, species-typical behavior without reference to the organism's representation of the relationships between the stimulus and the organism's life goals and behavior. It is suggested that the gerbil's concept of confinement and escape defines the stimulus and response classes of the escape reflex and produces the correlation that identifies the reflex as a reflex.

How might the gerbil's escape-reflex concept be depicted? A gerbil's reaction to being enclosed in a strange space is to engage in maneuvers that will expand the space. It is very likely that these resist-confinement reactions are relevant to the life goals of the Mongolian gerbil and, in fact, plausible adaptive functions may be proposed for behavior which operates to remove an animal from a confined space. This life goal is served by a conceptual organization in which an hierarchy of escape reactions are associated with a class of containment stimuli. The organization of the hierarchy remains to be empirically affirmed, however, personal observation indicates an organization of this nature: explore the surface area first, explore areas accessed by digging second, explore areas accessed by jumping third, explore areas accessed by climbing fourth and also, in conjunction with these maneuvers, make attempts to squeeze through an opening first and gnaw at the barrier second.

Since the environmental context is represented within the concept, there is, presumably, an organization in the way it is selectively perceived. Although this perceptual scheme is yet to be empirically determined, observation indicates that those aspects of the environment that may be incorporated into escape behavior (i.e., its digability, its climbability, its gnawability) receive selective attention in the conditions in which an escape reflex is evoked.

Therefore, given the present arguments, it is suggested that an escape-reflex concept is a representation of the relationship between a life goal of resisting confinement, a hierarchy of escape responses, and a set of environmental situations that both: 1. create the impression of confinement and 2. are incorporable into an escape strategy.

## LITERATURE CITED

Blanchard, R.J., Blanchard, D.C. and Hori, K. 1989. An ethoexperimental approach to the study of defense. *In* R.J. Blanchard *et al.* (eds.), *Ethoexperimental approaches to the study of behavior.* Kluwer Academic Publishers, Boston, MA, 114-136.

Dewey, J. 1896. The reflex arc concept in psychology. *Psychological Review,* 3:357-370.

Gibson, J.J. 1960. The concept of the stimulus in psychology. *American Psychologist,* 15:694-703.

Koffka, K. 1935. *Principles of Gestalt Psychology.* Harcourt and Brace Publishers, New York, NY.

Kotary, L., Cleary, T. and Drews, D. 1990. The development of escape behaviors in Mongolian gerbils. *J PA Acad Sci,* 64(3):116-120.

Skinner, B.F. 1935. The generic nature of the concepts of stimulus and response. *Journal of General Psychology,* 12:40-65.

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# RESEARCH NOTE

## DOES THE COLOR PATTERN OF TWO SPECIES OF TURTLES IMITATE DUCKWEED?[1]

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### ABSTRACT

**We propose that the spotted carapacial pattern of both Blanding's turtle (*Emydoidea blandingii*) and the spotted turtle (*Clemmys guttata*) imitates duckweed (family Lemnaceae). This relationship may be selectively advantageous in reducing vulnerability to predation. The range of duckweed is somewhat related to the range of both species of turtles, primarily in the east central United States. Duckweed extends northward beyond the range of both turtles and becomes quite rare along the western Great Plains where neither species of turtle occurs. Scarcity of lentic habitat, interspecific competition, winter temperatures and habitat suitability may also limit the distribution of both species of turtles. The presence of duckweed in some portions of the range of these two species of turtles may have some bearing on their color pattern but other factors in other areas may limit their distribution. [J PA Acad Sci 66(1):39-42, 1992]**

### INTRODUCTION

Natural selection is an important factor influencing the colors and patterns that animals evolve to avoid predators (Cott 1940, Endler 1978). Numerous studies bear out the adaptive significance of color and pattern in animals (see reviews by Curio 1976 and Endler 1978); however, little is known of the determinants of these traits (Endler 1978). Camouflage is imitation by an animal of its environment, either whole or in part (Pasteur 1982). Both Blanding's turtle *(Emydoidea blandingii)* and the spotted turtle *(Clemmys guttata)* possess a conspicuous yellow-spotted black carapace (Conant 1975). Pope (1967) proposed that the carapacial pattern of *E. blandingii* "blends with . . . aquatic vegetation." We propose that this cryptic pattern may be selectively advantageous in both *E. blandingii* and *C. guttata* as their carapacial patterns imitate the color and form of the floating-leaved duckweeds (family Lemnaceae, primarily *Lemna* and *Spirodela*). Herein, we compare the distribution of both species of turtles with that of the Lemnaceae of North America.

### MATERIALS AND METHODS

The distribution of duckweed was determined by compiling detailed distribution maps from published works. Those references which did not contain detailed distribution maps (i.e. contained a brief statement or map that portrayed duckweed as merely present or absent within a particular state or province) were not used. It was felt that such information was too scant to accurately portray distribution. The distribution of Blanding's and spotted turtles was determined by consulting major reviews of the respective species (Ernst 1972, McCoy 1973).

### RESULTS AND DISCUSSION

*Emydoidea blandingii* ranges from Nebraska east to southern Ontario and south to central Illinois. Disjunct

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Present Addresses:
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populations occur in Nova Scotia, New York, Massachusetts, and other parts of New England (McCoy 1973). This type of geographic distribution is characteristic of biota inhabiting the Prairie Peninsula which occurred following the retreat of Wisconsin glaciation (Smith 1957). The distribution of floating-leaved plants in the duckweed family is partially congruent with the distribution of *E. blandingii* in that duckweed becomes scarce or absent at the western edge of the Great Plains (Barkley 1977, Van Bruggen 1976). Duckweed is scarce in the westernmost portion of Kansas (Gates 1940, Barkley 1977). *Emydoidea blandingii* does not occur west of central Nebraska (McCoy 1973). The distribution of duckweed in Colorado (Harrington 1954) and Wyoming (Porter 1965) is not extensive. Within the central portion of the Blanding's turtles range (Indiana, Illinois, and Michigan), duckweed is common (Deam 1940, Mohlenbrock 1970, Voss 1972). Duckweed is commonly found beyond the northern and southern borders of *E. blandingii* range in Minnesota and Missouri (Lakela 1965, Steyermark 1963) indicating that the distribution of this and other syntopic turtle species may be affected by other factors, including: interspecific competition (Gutzke and Packard 1987), temperature that overwintering hatchlings are exposed to (St. Clair and Gregory 1990), and overall habitat suitability (Ross and Anderson 1990). Duckweeds extend into Canada (Budd 1951) north to the limit of trees along Hudson's Bay, east to southern New Brunswick (Hinds 1986), and Nova Scotia and west to Alaska (Polunin 1959, Porslid 1980). Preston and McCoy (1971) and McCoy (1973) suggest a subrecent extinction in peripheral parts of the range of *E. blandingii* because of the northward retreat of marshland habitat since Wisconsin glaciation.

FIGURE 1. The stippled pattern shows the general distribution of the Lemnaceae (primarily *Lemna* sp. and *Spirodela* sp.) in North America. The composite range of the Blanding's turtle and spotted turtle is designated with diagonal lines. The range of the Lemnaceae extends north to the limit of trees in northern Canada. In Colorado, few scattered records exist; the Lemnaceae are absent from northwestern peninsular Florida. In western and eastern West Virginia, scattered records (N = 8) exist and in the Blue Ridge province, the Lemnaceae are sporadic. For applicable references, consult the text.

*Clemmys guttata* occurs from northwestern Illinois east to Maine and south to northern Florida (Ernst 1972). The species' range along the Coastal Plain of North and South Carolina, and Georgia (Martof *et al.* 1980) closely follows that of the Lemnaceae (Harrison and Beal 1964, Radford et al. 1968, Jones and Coile 1988). This may be related to the rarity of natural lentic habitat, necessary for the establishment of duckweed above the Fall Line in these states. Other species of turtles have been shown to be limited in distribution by the Fall Line (Tinkle 1959). *Clemmys guttata* is absent from the southern Appalachian Mountains (Ernst and Barbour 1972), but enters West Virginia at its easternmost county (Green and Pauley 1987). Duckweeds are essentially absent from much of the Appalachian Mountains in Pennsylvania (Wherry *et al.* 1979), West Virginia (Strausbaugh and Core 1978), and from the Blue Ridge province [Virginia southwest to Georgia (Wofford 1989)]. *Clemmys guttata* is absent from the Allegheny Plateau and Allegheny Mountains of Pennsylvania (McCoy 1982) where duckweed is absent. Duckweeds are absent from hilly unglaciated southeastern Ohio (Cusick and Silberhorn 1977) where both species of turtles are conspicuously absent (Conant 1951). However, in Indiana where duckweed is widely distributed (Deam 1940), *C. guttata* is limited to the northern portion of the state (Minton 1972). The spotted turtle is present throughout most of southern Michigan where duckweed is similarly distributed (but absent from the easternmost portion of the lower peninsula), but duckweed is also found in northern Michigan (Voss 1972) where *C. guttata* is absent (Harding and Holman 1990). In Florida, the turtle is present in disjunct populations in the northcentral portion (Iverson and Etchberger 1989) and the duckweeds are too (Clewell 1985). However, duckweed in abundant in the wetlands of southern Florida (Long and Lakela 1971) where *C. guttata* is conspicuously absent. Clearly, other factors affect the distribution of *C. guttata.*

In summary, in some portions of the range, the presence or absence of duckweed may have some bearing on the color pattern of *E. blandingii* and *C. guttata.* This hypothesis is circumstantially supported by the general congruence of each turtles distribution with that of the Lemnaceae. However, in other areas, other factors may be more critical and thus limit the species distributions. As Portmann (1959) noted, "color adaptation itself needs no proof, but its survival value does." Assuming that the color pattern of *C. guttata* and *E. blandingii* does mimic duckweed, what possible advantage would this confer to the turtles? Hatchlings of both species are extremely vulnerable to predation (Ernst 1976, Congdon *et al.* 1983, Frazer *et al.* 1990). Since juveniles and sub-adults of both species are patterned like adults, it is possible that the duckweed-like pattern effectively conceals younger turtles from would-be predators. The annual probability of survival increases rapidly as turtles grow (Frazer *et al.* 1990), but adults are still vulnerable to predation (Seigel 1980, Emmons 1989). An alternative explanation is that the color pattern conceals the turtles from potential prey items. Since both species of turtles prey on small aquatic invertebrates such as snails, molluscs, dragonfly larvae, crayfish (Ernst 1976) or vertebrates (small fish, frogs, etc., Lagler 1943), perhaps concealing color patterns lessen the visibility of the turtles to these potential prey items.



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## LITERATURE CITED

Barkley, T.M. (ed.) 1977. Atlas of the flora of the Great Plains. Iowa State Univ. Press. Ames. 60pp.

Budd, A.C. 1951. A key to the plants of the farming and ranching areas of the Canadian prairies. Exp. Farms Serve. Canada Dept. Agric. 339pp.

Clewell, A.F. 1985. Guide to the vascular plants of the Florida panhandle. Florida State Univ. Press. Tallahassee. 605pp.

Conant, R.C. 1951. The reptiles of Ohio. Univ. Notre Dame Press. Notre Dame, Indiana. 284pp.

_____. 1975. A field guide to reptiles and amphibians of eastern and central North America. Houghton Mifflin Co. Boston 429pp.

Congdon, J.D., D.W. Tinkle, G.L. Breitenbach and R.C. van Loben Sels. 1983. Nesting ecology and hatching success in the turtle *Emydoidea blandingi.* Herpetologica 39:417-429.

Cott, H.B. 1940. Adaptive colorations in animals. Oxford Univ. Press. N.Y. 508pp.

Curio, E. 1976. The ethology of predation. Springer-Verlag. New York. 250pp.

Cusick, A.W. and G.M. Silberhorn. 1977. The vascular plants of unglaciated Ohio. Bull. of the Ohio St. Univ. Biol. Surv. 5. Columbus. 157pp.

Deam, C.C. 1940. Flora of Indiana. W.B. Burford Printing Co. Indianapolis. 1236pp.

Emmons, L.H. 1989. Jaguar predation on chelonians. J. Herpetol. 23:311-314.

Endler, J.A. 1978. A predator's view of animal color patterns. Evol. Biol. 11:319-364.

Ernst, C.H. 1972. *Clemmys guttata.* Cat. Am. Amphib. Reptiles. 124:1-2.

_____. 1976. Ecology of the spotted turtle, *Clemmys guttata* (Reptilia, Testudines, Testudinidae), in southeastern Pennsylvania. J. Herpetol. 10:25-33.

Frazer, N.B., J.W. Gibbons, and J.L. Greene. 1990. Life tables of a slider turtle population. In, J.W. Gibbons (ed.), Life history and ecology of the slider turtle. Smithsonian Institution Press. Washington, D.C. pp. 183-200.

Gates, F.C. 1940. Flora of Kansas. Kansas Agric. Exp. Stn. Topeka. 266pp.

Green, N.B. and T.K. Pauley. 1987. Amphibians and reptiles in West Virginia. University of Pittsburgh Press. 241pp.

Gutzke, W.H.N. and G.C. Packard. 1987. The influence of temperature on eggs and hatchlings of Blanding's turtles, *Emydoidea blandingii.* J. Herpetol. 21:161-163.

Harding, J.H. and J.A. Holman. 1990. Michigan turtles and lizards. Michigan State Univ., Coop. Ext. Serv. 94pp.

Harrington, H.D. 1954. Manual of the plants of Colorado. Sage Books. Denver. 666pp.

Harrison, D.E. and E.O. Beal. 1964. The Lemnaceae (Duckweeds) of North Carolina. J. Elisha Mitchell Scientific Society. 80:12-18.

Hinds, H.R. 1986. Flora of New Brunswick. Primrose Press. Fredericton, New Brunswick. 460pp.

Iverson, J.B. and C.R. Etchberger. 1989. The distributions of the turtles of Florida. Florida Scientist. 52:119-144.

Jones, S.B. and N.C. Coile. 1988. The distribution of the vascular flora of Georgia. University of Georgia. Athens. 230pp.

Lagler, K.F. 1943. Food habits and economic relations of the turtles of Michigan with special reference to fish management. Am. Midl. Nat. 29:257-312.

Lakela, O. 1965. A flora of northeastern Minnesota. Univ. of Minnesota Press. Minneapolis. 541pp.

Long, R.W. and O. Lakela. 1971. A flora of tropical Florida. Univ. of Miami Press. Coral Cables. 962pp.

Martof, B.S., W.M. Palmer, J.R. Bailey, and J.R. Harrison, III. 1980. Amphibians and reptiles of the Carolinas and Virginia. University of North Carolina Press, Chapel Hill. 264pp.

McCoy, C.J. 1973. *Emydoidea, E. blandingii.* Cat. Am. Amphib. Reptiles 136:1-136:4.

McCoy, C.J. 1982. Amphibians and reptiles in Pennsylvania. Carnegie Museum of Natural History, Special Publ. 6. 1-91.

Minton, S.A., Jr. 1972. Amphibians and reptiles of Indiana. Indiana Acad. Sci. Monograph Number 3:1-346.

Mohlenbrock, R.H. 1970. The illustrated flora of Illinois. Flowering plants. Flowering rush to rushes. Southern Illinois Univ. Press. Carbondale and Edwardsville. 272pp.

Pasteur, G. 1982. A classificatory review of mimicry systems. Ann. Rev. Ecol. Syst. 13:169-199.

Polunin, N.V. 1959. Circumpolar artic flora. Oxford at the Clarendon Press. 514pp.
Pope, C.H. 1967. Turtles of the United States & Canada. A.A. Knopf. N.Y. 337pp.
Porslid, A.E. 1980. Vascular plants of the continental Northwest Territories, Canada. National Museum of Natural Sciences. Ottawa. 667pp.
Porter, C.L. 1965. A flora of Wyoming. Agric. Exp. St. Univ. Wyoming. Bull. 434. 88pp.
Portmann, A. 1959. Animal camouflage. Univ. Michigan Press. Ann Arbor. 111pp.
Preston, R.E. and C.J. McCoy. 1971. The status of *Emys tweentei* Taylor (Reptila: Testudinidae) based on new fossil records from Kansas and Oklahoma. J. Herpetol. 5:23-30.
Radford, A.E., H.E. Ahles, and C.R. Bell. 1968. Manual of the vascular flora of the Carolinas. The Univ. of North Carolina Press. Chapel Hill. 1183pp.
Ross, D.A. and R.K. Anderson. 1990. Habitat use, movements, and nesting of *Emydoidea blandingii* in central Wisconsin. J. Herpetol. 24:6-12.
St. Clair, R.C. and P.T. Gregory, 1990. Factors affecting the northern range limit of painted turtles *(Chrysemys picta)*: Winter acidosis or freezing. Copeia 1990:1083-1089.
Seigel, R.A. 1980. Predation by raccoons on diamondback terrapins, *Malaclemys terrapin tequesta.* J. Herpetol. 14:87-89.
Smith, P.W. 1957. An analysis of post-Wisconsin biogeography of the prairie peninsula region based on distribution phenomenon among terrestrial vertebrate populations. Ecology 38:205-218.
Steyermark, J.A. 1963. Flora of Missouri. The Iowa State Univ. Press. Ames. 1725pp.
Strausbaugh, P.D. and E.L. Core. 1978. Flora of West Virginia. Seneca Books. Inc. Grantsville, WV. 1079pp.
Tinkle, D.W. 1959. The relation of the fall line to the distribution and abundance of turtles. Copeia 1959:167-170.
Van Bruggen, T. 1976. The vascular plants of South Dakota. The Iowa State Univ. Press. Ames. 538pp.
Voss, E.G. 1972. Michigan flora. Part 1. Gymnosperms and monocots. Cranbrook Institute of Science. Bloomfield Hills, MI. 488pp.
Wherry, E.T., J.M. Fogg Jr., and H.A. Wahl. 1979. Atlas of the Flora of Pennsylvania. Morris Arboretum of the University of Pennsylvania. Philadelphia. 390pp.
Wofford, B.E. 1989. Guide to the vascular plants of the Blue Ridge. Univ. of Georgia Press. Athens and London. 384pp.

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# COMMENTARY

## A TRAINING COURSE FOR LEAD ABATEMENT WORKERS AND SUPERVISORS[1]

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**ABSTRACT**

**Several state agencies, require and the Department of Housing and Urban Development (HUD) recommends, that workers and supervisors conducting lead abatement have proper training. The purpose of this training is to minimize public health hazards, prevent environmental contamination, and reduce the number and severity of injuries, accidents and lead exposure to site personnel. A model training program must provide theory and practical aspects to adequately meet these goals. This paper describes an initial lead abatement training program for workers and supervisors. The described course(s) should provide a model for standardization of lead abatement training. An outline of this training course(s) is presented and discussed.**

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### INTRODUCTION

Lead-based paint (LBP) abatement is developing as a "new" hazardous industry in the United States. Although this industry has existed for decades, many consider the industry "new" as a result of recent findings that document a hazard with traditional abatement practices, recognition that home improvement and home maintenance can have harmful effects for workers and occupants, and an increasing awareness of the health effects of exposure to sensitive members of the population (e.g. young children), especially at low level concentrations (Holmess and Nethercott, 1988; Schwartz, *et al.* 1990; Department of Housing and Urban Development - HUD, 1991; Farfel and Chisolm, 1991; Lange, *et al.* 1991; Lange, 1991). Several local governments (e.g. City of Baltimore), states (e.g. Massachusetts Department of Labor and Industry, 1988) and the Department of Housing and Urban Development (HUD) (City of Baltimore, 1987; Massachusetts Department of Labor and Industries, 1988; HUD, 1990) have developed training requirements for lead abatement workers (deleaders), supervisors, contractors and inspectors. The lead abatement training requirements are evolving similarly as was training for asbestos abatement workers, supervisors, inspectors, management planners and project designers. However, unlike asbestos, lead abatement requirements are initially being developed and established by state and local agencies with the federal government only recently providing guidance. The state standards have resulted in varying and inconstant practices and procedures being issued. Regardless of the lead regulation examined, each statute provides that the purpose of training is to protect the worker, public and environment.

This paper describes a model training course for lead abatement workers and supervisors. The course curriculum meets or exceeds training requirements established or proposed by the states of Connecticut Department of Health Services, Maryland and Massachusetts and HUD (Connecticut, 1990; Massachusetts Department of Labor and Industries, 1988; Maryland Department of the Environment, 1983; HUD, 1990). The course objectives are to provide deleaders with an introduction to safe work practices and prevention of occupational diseases (e.g. prevention of lead poisoning), accidents, injuries and prevention of exposure to the public, control of environmental contamination and protection of sensitive members of the population. This course may be a useful model for regulatory agencies that are establishing lead training requirements.

### DISCUSSION

The hazards associated with activities involving lead, including lead abatement, have been recognized for decades (Zimmer, 1961; Rieke, 1969; Holmess and Nethercott, 1988; HUD, 1988; Booker, 1988; Lange et. al., 1991). Most workers in the lead abatement industry, which include the construction trades, have little or no training on respiratory devices, health effects, medical surveillance, and appropriate work practices. The reduction of injuries and disease in the lead abatement industry can be accomplished through effective training programs. A lead abatement

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training program must include practical and theoretical aspects of instruction.

The model worker course is three days in length, with approximately one day of hands-on training; while the supervisor course includes all the worker training plus one additional day of instruction. The supervisor training program described in this paper is also applicable to contractors and could possibly be designated as a supervisor/contractor course. However, it may be beneficial for contractors to have an additional day of training specifically applicable to contracting activities (e.g. workers' compensation, management, employee relations, establishing medical surveillance).

## INTRODUCTION AND OVERVIEW

Course participants will be informed on the location of sanitary facilities, phone, emergency exits, course schedule and available refreshments. A brief overview of the course, types (including use, identification, physical characteristics and appearance) of lead containing materials (Subramanian, *et al.* 1991; Spangenberg and Stower, 1988) and history (National Institute of Building Sciences - NIBS, 1988) of lead abatement in the United States will be presented. Students/participants will be encouraged to ask questions and participate in a dialogue with instructors. Completion and participation in all lectures, practical exercises and home work assignments are required for issuance of a course certificate.

## HEALTH EFFECTS

A discussion of diseases related to lead abatement will be presented. This lecture will emphasize routes of exposure, dose response relationships, acute vs. chronic exposure, body storage, excretion, metabolism, (Lange, 1991) differences between adults, children (Chisolm, *et al.* 1985), and other sensitive members of the population, (Bellinger *et al.* 1991; Office of Technology Assessment, 1985; Schwartz, *et al.* 1990), and signs and symptoms of lead poisoning (Saurai, *et al.* 1974; Rekus, 1988; Zenz, 1988; Environmental Protection Agency - EPA, 1984; Rom, 1983; Rosenstock and Cullen, 1986; Feldman, 1978; Charney, *et al.* 1980; Karam and Beck, 1990). The physiological effects of lead on various organ systems (e.g. kidney, blood, nervous, reproductive, musculo-skeletal) including blood concentration and corresponding health concerns, will be used to explain its toxicology. The lack of a safe level for lead will be emphasized. A brief discussion of other abatement health hazards (e.g. mercury) and the potential health effects from these substances will be included in this lecture to alert and inform deleaders (Agoes, *et al.* 1990; Centers for Disease Control, 1990; Lange, *et al.* 1991). Permissible Exposure Limits (PEL) and Threshold Limit Values (TLV) for lead, asbestos and other substances that are associated with abatement will be discussed. The use of the PEL and the TLV as a guide for disease prevention will be emphasized (Occupational Safety and Health Administration - OSHA, 1990; American Conference of Governmental Industrial Hygienists - ACGIH, 1991). A brief discussion of monitoring techniques will be used to orient participants on the proper practices and procedures for collecting information on exposure concentrations for occupational and public health prevention (Lange, 1991; Lange, 1991a).

## MEDICAL SURVEILLANCE AND MONITORING

A review will be presented of the Occupational Safety and Health Administration (OSHA), HUD and State requirements (e.g. Maryland, Connecticut and Massachusetts) for medical surveillance of lead abatement workers and persons occupationally exposed to lead (Connecticut Department of Health Services, 1990; OSHA, 1990a; HUD, 1990, Maryland Department of Licensing and Regulations, 1984; Maryland Department of the Environment, 1983; Massachusetts Department of Labor and Industries, 1988). Emphasis will be placed on the importance of complete medical examinations (physical examinations) and periodic surveillance (Lange, et. al. 1991a; Medius, 1986; Connecticut Department of Health Services, 1990; Maryland Department of the Environment, 1983). Medical surveillance criteria (e.g. medical history, evaluation of organ systems, with emphasis on the cardiovascular, pulmonary, oral cavity, kidneys, reproductive, neurological, gastrointestinal, blood, and musculo-skeletal, past and future exposures, personal habits and ergonomic factors) will be presented. Medical requirements related to using personnel protective equipment (PPE) (Harber, 1984; Lange, *et al.* 1991a) will be included in this lecture. The reason(s) and importance of blood pressure measurements, blood samples (blood lead level, hemoglobin, hematocrit, red blood cell indices, peripheral blood smear, zinc protoporthyrin - ZPP or free erythrocyte protoporphryn - FEP, blood nitrogen, serum creatinine), urinalysis (including microscopic examination) and pregnancy testing, if applicable will be presented. Periodic medical monitoring criteria, frequency and removal requirements will be addressed (Sheldon, *et al.* 1986; Secchi and Alessio, 1974; Tomokani and Ogata, 1976). Regulatory requirements of termination medical exams, employee notification, multiple physician review, and medical laboratory selection will be presented (Massachusetts Department of Labor and Industries, 1988; OSHA, 1990a). The advantages and disadvantages of chelation therapy will be reviewed (Batuman, *et al.* 1989; Zenz, 1988). Emphasis will be placed on preventative practices and appropriate medical surveillance as a mechanism of minimizing disease. An example of blood lead level changes over time at various exposure concentrations will be presented (Lange, 1991). The medical surveillance and monitoring program as a living document that requires modification and change over time will be a continual theme in this lecture. (Lange, *et al.* 1991a).



## PERSONAL HYGIENE AND SAFETY FACTORS, OTHER THAN LEAD ABATEMENT

The importance of personal hygiene practices in the workplace as a health issue for individuals will be the primary focus of this lecture.

Participants will be instructed on the importance of proper personal hygiene (Olishifski, 1987) and proper entry into and exit from the work area. Smoking, drinking, eating, applying cosmetics and improper decontamination practices will be used to emphasize preventable routes of exposure and its prevention.

Accidents and injuries during lead abatement often result in a greater number of worker's compensation claims than lead related diseases. This occurrence warrants the amount of time devoted to safety factors (accident prevention) and practical first aid training (Lange, 1991a). Potential exposure to the deleader's family and general public from improper decontamination, personal hygiene and work practices will be included in this lecture.

A discussion of accident prevention will include heat stress (Fuller and Smith, 1981; National Institute of Occupational Safety and Health - NIOSH, 1986), cold stress, (hypothermia and frostbite) (American Conference of Governmental Industrial Hygienists, 1991; Zenz, 1988) slips, trips and falls, scaffold safety, electrical hazards, cuts, confined space environments (Pettit and Linn, 1987; Lange, 1991a), air contaminants other than lead (OSHA, 1989; Plummer, *et al.* 1987), burns (chemical, electrical and heat), and emergency transport (American Red Cross, 1988; Lange, 1991a). The importance of fire prevention, use of fire extinguishers and emergency planning will be discussed (Hammer, 1985). Hearing prevention practices (Lord, *et al.* 1980; Lempert, 1984; Zenz, 1988), as related to lead abatement activities, will be included in this lecture.

## HAZARD COMMUNICATION

This lecture will present OSHA's hazard communication standard (29 CFR 1910.1200). Employee rights and employer responsibilities will also be discussed (Kendig, 1990). A presentation of material safety data sheets (MSDS), their use, interpretation and required dissemination will be included. Review and discussion of a MSDS will be used to demonstrate to participants the information available from this resource (Brady, 1987; US Department of Labor, 1981).

## REGULATORY CONSIDERATIONS

An overview of regulatory requirements for lead abatement, transport of hazardous materials and disposal of abatement waste will be presented. This presentation will include a discussion of HUD guidelines (Federal Register 55: 14557), Maryland Department of Environment, (Title 26, subtitle 02), Massachusetts Department of Labor and Industries (454 CMR 22.00), Connecticut Department of Health (Sections 19a-111-1 through 19a-111-11), OSHA Standards (employee access to medical records - 29 CFR 1910.20; personal protective equipment - 29 CFR 1910.132, .133, .134, .135, .136 and .137; fire extinguishers 29 CFR 1910.157; toxic and hazardous substances 29 CFR 1910.1000; Lead Standard 29 CFR 1910.1025), Resource Conservation and Recovery Act (RCRA), Hazardous Materials Transportation Act (HMTA) and state hazardous waste and disposal requirements (Lange, 1991; Lange, 1991b; Pennsylvania Department of Environmental Resources, 1988). Emphasis will be placed on the HUD and state requirements, especially as related to work practices, and notification procedures. Recommendations by the National Institute of Occupational Safety and Health (NIOSH) on safe lead abatement practices and personal protection procedures will be reviewed during this lecture.

## PERSONAL PROTECTIVE EQUIPMENT

This lecture will present a discussion on personal protective equipment (PPE), which will include respiratory devices, clothing, face/eye protection, footwear and gloves (Lange, 1991a; Rajhans and Blackwell, 1985; Rork, et. al. 1984; NIOSH/OSHA/USCG/EPA, 1985; Bollinger and Schultz, 1987; Schwope, *et al.* 1987). The importance of PPE as a mechanism to protect the individual worker will be emphasized. A discussion of respirator selection, limitations, factors that alter fit, maintenance, repair/replacement, cleaning, use and decontamination practices will be presented. Procedures for handling, use, storage and selection of non-disposable clothing will be discussed. OSHA's respiratory protection (29 CFR 1910.134) and lead (29 CFR 1910.1025) standards; and respiratory requirements described in HUD, and the states of Maryland, Connecticut and Massachusetts will be reviewed in this lecture. Since abatement often involves exposure to numerous hazardous substances implementation and selection of PPE programs for multi-hazard environments will be discussed.

## PRE-WORK ACTIVITIES, WORK AREA PREPARATION AND ENGINEERING CONTROLS

An overview of the sequence of lead abatement activities will be presented (Lange and Spence, 1990; Chisolm, 1986; Rey-Alvarez and Menke, 1987). Pre-work activities to be discussed include: water, electrical and sanitary hookup; evaluation of preexisting conditions, notification of residents and agencies; posting of signs, removal of belongings, establishing work locations and developing emergency plans (HUD, 1990). Coordinating activities with residents, owners and related entities will be discussed.

This lecture will include a discussion of building a decontamination station, contaminant barriers, ventilation system

control and lockout, entrance and exit procedures, and methods of preventing the release of hazardous pollutants. Indoor and outdoor abatement (e.g. trim, surfacing, soffit, facia and soil cleanup) activities will be included in this presentation. The appropriate use of negative air machines, HEPA vacuums and decontamination air locks will be discussed.

## LEAD ABATEMENT WORK PRACTICES

The advantages, disadvantages and applicability of various lead abatement work practices will be presented (Farfel and Chisolm, 1991; HUD, 1990). Practices that will be discussed include removal, replacement, encapsulation and reversal. Specific techniques (e.g. open flame burning) that are prohibited by state regulations or discouraged by standard practice will also be discussed. Hazards created by using these techniques will be used to demonstrate their inappropriateness (Fischbein *et al.* 1981). Procedures for decontamination and disposal of contaminated soil are included in this lecture (Solomon and Hartford, 1976; HUD, 1990; NIBS, 1988; Ter Haar and Aronow, 1974).

Safety system design and the importance of pilot studies to evaluate the effectiveness of a specific technique for a specific situation will be a recurrent theme throughout this lecture (Farfel and Chisolm, 1991). A case study will be presented to demonstrate the importance of pilot studies, individualized approaches and determining the effectiveness of a technique.

## DECONTAMINATION AND CLEANING THE WORK AREA

Decontamination and cleanup practices will be presented (NIBS, 1988; HUD, 1990). The importance of daily decontamination and maintenance of a clean work area will be emphasized. Methods of decontamination and cleanup will be presented through a step by step approach. Sample problems will be presented to provide participants with practice in preparing cleaning solutions. The importance of preventing the spread of contamination through proper cleaning techniques is included in this lecture.

## DISPOSAL PRACTICES

A discussion of bagging, drumming, transporting and disposal of lead abatement waste will be presented (West, 1986; US Congress, Office of Technical Assessment, 1986). Techniques of storing and preparing waste for transport to a disposal site will be reviewed (NIBS, 1988). Participants will be provided with examples of effective treatment(s) of waste water and decontamination of equipment (NIOSH/OSHA/USCG/EPA, 1985; NIBS, 1988).

Toxicity Characteristic Leaching Procedure (TCLP) testing requirements, sample collection and its use in determining hazardous waste characteristics will be presented (Tinklenberg, 1988; NIBS, 1988). Federal and state hazardous waste standards will be included in this lecture (Lange, 1991b; Pennsylvania Department of Environmental Resources, 1988a).

## PERSONAL PROTECTIVE EQUIPMENT - PRACTICAL

Practical training will consist of cleaning, assembly, donning and doffing, basic repair, disinfection, and fit testing practices and procedures of respirators (Lange, 1991a). Class of respirators to be included in this practice session include negative pressure half mask air purifying and powered air purifying. All participants will be required to participate in fit testing, disassembling, cleaning, disinfecting and reassembling of a half mask air purifying respirator. The fit testing protocol will be as described in the OSHA lead standard (29 CFR 1910.1025).

Proper donning and doffing of protective clothing and shoe covers will be included in this practice session. All participants will have the opportunity and time to evaluate and use other forms of personal protective equipment (e.g. safety glasses, face shields). This will allow participants to obtain a practical understanding, proper use, maintenance, selection criteria, and some supervised experience with PPE.

## FIRST AID PRACTICAL

Injuries are a common occurring event at abatement sites (Lange, *et al.* 1991a and Lange, 1991a). A basic knowledge in first aid can provide immediate treatment for severe and minor injuries, as well as providing a basic premise of accident prevention. It should be emphasized to participants that this training is introductory, and all deleaders should attend and successfully complete at a minimum, an American Red Cross Standard First Aid Course. This section should be taught by a qualified First Aid Instructor.

The basic skills presented and practiced in this session include: measuring a pulse, blood restriction pressure points, bandages (bleeding, arm sling, ankle bandage), treatment of burns, emergency rescue, rescue breathing, heat stress prevention, recognition, monitoring and treatment (heat stroke, heat exhaustion and heat cramps), and cold stress prevention, recognition and treatment (frostbite and hypothermia). Signs and symptoms of preventable injuries (e.g. heat stress) and first aid awareness will be emphasized during this practice session (American Red Cross, 1988; Lange, 1991).

## PREPARING THE WORK AREA - PRACTICAL

The class should be divided into small groups and each group, with assistance from an instructor, will build a decontamination station, a critical barrier and post proper signs (NIBS, 1988). Participants will then critique their work, and instructors will provide practical suggestions arising from problems experienced during this exercise.



## LEAD ABATEMENT WORK PRACTICES - PRACTICAL

Various work stations should be established for instruction and practice using heat guns, a HEPA vacuum sander, wet scraping, and touch up work with chemical agents. Instructors should provide guidance for the practical use of this equipment and its appropriate application.

Surfaces that should be available for practice sessions include window frames, doors and walls. Each work station should have encapsulation materials that participants can use after they complete their abatement activity. A sufficient number of experienced instructors must be available to assist participants and provide feedback on their work practices. Paint that is applied on surfaces for practice removal must not contain lead or mercury above current regulatory limits (Lange, *et al.* 1991). Safe work practices will be emphasized by instructors during demonstrations, and they will provide a critique of the participants performance.

## DECONTAMINATION AND CLEANUP - PRACTICAL

Participants will be required to prepare cleaning solutions and demonstrate proficiency using cleaning/decontamination equipment (e.g. HEPA vacuum) (NIBS, 1988; Maryland Department of the Environment, 1983). The areas/stations previously abated will be used for cleaning/decontamination activities. Practical exercises in disposal of cleaning solutions and changing HEPA vacuum bags will be included in this practice session. Participants will properly decontaminate equipment and transport it through the decontamination station. The importance of proper cleaning and prevention of environmental contamination will be emphasized.

## STRIPPING AGENT DEMONSTRATION

The demonstration of a stripping agent (e.g. Peel Away) (Spec data, 1988) should be conducted on a door or wood trim surface. This should be performed in two phases. The first phase will demonstrate the preparation of this material and application on the surface; while the second phase will demonstrate its removal, proper containment, storage and methods of disposal (e.g. drums). For the second phase, an instructor must apply the material a day in advance for a proper demonstration. This demonstration will provide participants with an understanding of practical and non-practical uses of on-site stripping. It should be emphasized to all participants, especially supervisors and contractors, that this demonstration may not be adequate to train personnel in the use of stripping agents. Additional practical training, beyond this demonstration, may be necessary for workers that have never used stripping agents. The work practice-practical session could incorporate hands-on training with stripping agents. However, the practical work session time period should be expanded if the use of stripping agents are included in this session. The initial use of stripping agents by workers at the lead-based paint abatement site will require strict supervision, regardless of the amount or type of training in this course. The site supervisor should determine a worker's competence before allowing unsupervised work with stripping agents. This can usually be accomplished in a day or two of hand-on field work. Many companies selling stripping agents will provide additional training for their particular product(s). The problems of disposal and health hazards associated with strippers will be emphasized.

## DEVELOPMENT OF A SITE HEALTH AND SAFETY PLAN

A brief presentation will be conducted on developing a site health and safety plan (SHSP) (also called a site safety and health plan-SSHP or site specific safety plan-SSSP) for a lead abatement project. Topics discussed will include: emergency contacts, safety training, emergency procedures, site control, heat and cold stress monitoring, fire safety, key personnel, medical surveillance and monitoring, PPE, decontamination set-up and procedures, emergency rescue, hazard evaluation, air monitoring and electrical hazards (Lange, 1991a; Sawyer, 1985).

The purpose of this lecture and work session is to familiarize participants (supervisors) with important practices, procedures, difficulties and preventive measures in establishing, implementing and developing a SHSP.

A hypothetical abatement project will be presented for the purpose of developing an outline of a SHSP. The class will be divided into small groups, three to five participants, for this exercise. Each group will briefly (2-5 minutes in length) present how they would implement their plan. Instructors and other class members will critique the implementation, and suggest practical problems and solutions associated with the plan.

The instructor(s) presenting this lecture should be familiar with SHSP's emergency response, and have practical experience in preparing an SHSP.

## LEGAL AND INSURANCE ISSUES

Legal issues to be included in this presentation include: contracts, hold harmless clauses, third party liability, toxic tort, negligence, and agency law (Steffen, 1977; Schaber and Rohwer, 1984; Findley and Farber, 1988; Feitshaws, 1991).

The complexity of abatement and the associated hazards impacting on legal issues should be discussed. Types of insurance and bonds that are necessary to conduct work should also be reviewed. Workers' compensation requirements and its differences among states should be included in this lecture (Hood and Hardy, 1984). This presentation should be conducted by an attorney experienced in either abatement or administrative law or an individual having training in the legal environment.

## CLEARANCE CRITERIA, AIR MONITORING AND TESTING FOR LEAD

A discussion and brief demonstration on final clearance wipe samples should be included in this presentation. Current acceptable values for final clearance should be discussed, as well as the issues related to these criteria (National Institute of Building Sciences, 1988; Holak, 1975; Campbell and Cross, 1976; Lauren *et al.* 1971; Coomber, 1975; Sayre and Wilson, 1970). The importance of using wipe samples (as compared to air samples) for final clearance should be addressed.

Air monitoring methods for determining exposure to site personnel should be presented. A demonstration of sampling equipment and some hands-on use should be included in this presentation. A case example of determining the number of personnel samples to be obtained, random selection of individuals to be monitored, monitoring frequency, sampling flow rates and duration of daily samples should be discussed (Farthing, 1982; Lioy and Lioy, 1983; Bar-Shalom, *et al.* 1975; Leidel, *et al.* 1977). This information should provide supervisors with adequate instruction to determine and interpret the lead exposure of deleaders.

Bulk sampling methods and analysis techniques for determining the concentration or presence of lead in various substrates should be presented (Lauren *et al.* 1971). A brief discussion of proper sampling, cleanup and repair should be discussed. A review of analytical techniques (laboratory, direct reading and colormetric) that can be used to detect and determine a lead concentration should be presented (Lauren *et al.* 1971; Campbell and Cross, 1976; Sayre and Wilson, 1970; Holak, 1975; Que Hee *et al.* 1985; Lange, 1991). The methods that should be included in this discussion at a minimum are atomic absorption spectrometry, X-Ray fluorescence and sodium sulfide spot test. Limitations, complexities of analysis, interpretation, interferences, and costs should be included in this presentation.

## LEAD INSPECTION AND MONITORING REPORTS

A brief presentation on understanding, interpreting and preparing lead inspection and monitoring reports will be discussed (NIBS, 1988; HUD, 1990). The importance of properly documented reports will be reviewed.

## SUPERVISORY TECHNIQUES

This presentation will focus on supervisory practices and techniques related to safety, proper work practices and minimizing exposure to hazardous materials. The importance of periodic site safety meetings (tail gate safety talks) and methods of presenting this information will be reviewed (US Department of the Interior, 1987).

## RECORDKEEPING AND DOCUMENTATION

Recordkeeping and notification requirements that are required by federal (e.g. OSHA), state (e.g. Maryland) and local (e.g. City of Baltimore) agencies will be reviewed. The importance of proper documentation as a legal record of project activity will be emphasized (Lange, 1991b). A case example of records and documents necessary for a typical project will be presented.

## UNDERSTANDING SPECIFICATIONS

Various types of specifications will be reviewed (Dunham and Young, 1975; Merritt, 1976). Pitfalls in specifications, incomplete documents and submittal requirements of bid documents will be discussed (Lange, 1991b). The use of various words (e.g. all), description of quantities, incorporated references or regulations and evaluation and interpretation of a specification will be presented (Association of Soil and Foundation Engineers, 1978). An example specification will be used to illustrate the various principles discussed in this lecture.

## COURSE REVIEW

The major topics of the three day worker course (e.g. health effects) will be reviewed. On day four, the review will focus on supervisory information. During this time period, participants will be encouraged to ask questions, and discuss issues relevant to lead abatement.

## HOMEWORK QUESTIONS

Homework questions should be provided to participants and discussed during the next class day. The purpose of these questions is to assist instructors in evaluating performance and provide participants with a review of major topics and concepts presented. These questions (five to ten per day), should be in essay form and oriented to the previous day's lecture material.

## EXAMINATION

A 50 and 100 question, multiple choice examination will be administered to workers and supervisors, respectively. The questions will be simple in nature and will focus on safe work practices, exposure, injury prevention, and public, occupational and environmental health considerations of



occupational and environmental health considerations of lead abatement. At the time of the worker examination, supervisors will proceed to an additional lecture track and a presentation on site health and safety will be conducted during this time slot. Topics specifically designed for supervisors are legal and insurance issues; clearance criteria, air monitoring and testing for lead; lead inspection and monitoring reports; supervisory techniques; recordkeeping and documentation; and understanding specifications.

## SUMMARY

This paper provides a model guide for the development of a lead abatement course. The length of time allotted for each lecture or practical session may be modified depending on the experience of the class. However, it is important that each participant receive adequate instruction on each topic. Practical areas of training should involve small groups with an experienced instructor. When work stations are used, there should be at least one instructor at each station. It may be possible to shorten the course for groups that have previous training (e.g. asbestos abatement) in related areas (e.g. respiratory protection). However, all topics and sessions must be presented, at least as a review, to ensure an adequate understanding of lead abatement practices, procedures and safety factors.

Individuals interested in becoming a contractor should attend the supervisor course. This training curriculum provides the basic information for the safe operation of lead abatement. However, contractors and upper management personnel should attend related courses (e.g. hazardous waste) to better understand practices and activities.

The minimum requirements for a course director are two years of practical experience in lead abatement, five years of teaching similar training programs (e.g. asbestos), and a masters degree in a science related discipline (e.g. engineering, environmental health, occupational health, industrial hygiene, chemistry, biological sciences). Since this industry is new, it is sufficient that the course director have only two years experience in the lead abatement industry; however, in the future this requirement should be increased.

The quality of this course is dependent on the instructor(s) qualifications and available materials for hands-on training. Each instructor should have sufficient training, practical experience and ability to present their topics, demonstration(s) and/or practical exercises. At a minimum, each instructor should have at least one year of practical experience in the lead abatement industry. Each participant must have a sufficient opportunity to use the various equipment and materials associated with this industry.

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## REFERENCES

Agoes, M.N., R.A. Etzel, R.G. Parrish, D.C. Paschal, P.R. Campagna, D.S. Cohen, E.M. Kilborbourne and J.L. Hesse. 1990. Mercury from interior latex paint. New England Journal of Medicine. 323:1096-1101.

American Conference of Governmental Industrial Hygienists. 1991. Threshold limit values for chemical substances and physical agents in the work environment for 1990-1991. Cincinnati, Ohio.

American Red Cross. 1988. Standard First Aid Workbook, American Red Cross. Washington, DC.

Association of Soil and Foundation Engineers. 1978. Professional liability and loss prevention manual. Silver Spring, MD.

Bar-Shalom, Y., D. Budenaers, R. Schainker and A. Segall. 1975. Handbook of statistical tests for evaluating employee exposure to air contaminants. National Institute of Occupational Safety and Health (NIOSH) (DHEW Pub. nos. 75-147). Cincinnati, OH.

Batuman, V., R.P. Wedeen, J.D. Bodgen, D.J. Balestra, K. Jones and G. Schodousky. 1989. Reducing lead content by chelatin treatment in chronic lead poisoning: an X-Ray fluorescence and bone biopsy study. Environmental Research 48:70-75.

Bellinger, D., A. Leviton, M. Rabinowitz, E. Allred, J. Needleman and S. Schoenbaum. 1991. Weight gain and maturity in fetuses exposed to low levels of lead. Environmental Resources 54:151-158.

Bollinger, N.J. and R.H. Schultz. 1987. NIOSH guide to industrial respiratory protection. U.S. Department of Health and Human Services. DHHS (NIOSH) Publication No. 87-116.

Booker, L.E. 1988. Lead exposure in a ship overhaul facility during paint removal. American Journal of the Industrial Hygiene Association. 49:121-127.

Brady, J.F., P.D. Shafer, J. Stevenson, J. Antopal, B. Kelly, I. Silverman and C. Hocker. 1987. Right-to-know Compliance Encyclopedia. Business and Legal Reports, Madison, CT.

Campbell, J.L. and L.A. Cross. 1976. Portable instrumentation for determination of lead in painted surfaces. Bulletin Environmental Contamination and Toxicology. 16:169-173.

Centers for Disease Control. 1990. Mercury exposure from latex paint-Michigan. Mortality and Morbidity Weekly Report. 39:125-126.

Charney, E., J. Sayre and M. Coulter. 1980. Increased lead absorption in inner city children: Where does the lead come from. Pediatrics 65:226-231.

Chisolm, J.J. 1986. Removal of lead paint from old housing: The need for a new approach. American Journal of Public Health. 76:236-237.

Chisolm, J.J. E.D. Melits and S.A. Quaskey. 1985. The relationship between the levels of lead absorption in children and the age, type and condition of housing. Environmental Research 38:31-45.

City of Baltimore. 1987. Abatement regulations for lead paint. Rules and Regulations Governing Housing Regulations. (July 1, 1987). Baltimore, MD.
Connecticut Department of Health Services. 1990. Lead poisoning and prevention and control regulations. Section 19a-111-1 through 19a-11. Hartford, CT.
Coomber, D.I. 1975. Radiochemical methods in analysis. Plenum Press, New York, NY. 298 pp.
Department of Housing and Urban Development. 1990. Lead-based paint; interim guidelines for hazard indentification and abatement in public and Indian housing. Federal Register 55:14557 (April 18, 1990).
Department of Housing and Urban Development. 1991. The Housing and Urban Development lead-based paint abatement demonstration. Washington, DC.
Department of Housing and Urban Development. 1988. The nature and extent of lead poisoning in children in the United States: a report to Congress. Agency for Toxic Substance and Disease Registry, Public Health Service. Atlanta, GA.
Dunham, C.W. and R.D. Young. 1975. Contracts, specifications and law for engineers. McGraw-Hill Book Company, New York, NY.
Environmental Protection Agency. 1984. Health effects assessment for lead. Cincinnati, OH. 43 pp.
Farfel, M.R. and J. Chisolm. 1991. An evaluation of experimental practices for abatement of residential lead-based paint: report on a pilot project. Environmental Research 55:199-212.
Farthing, W.E. 1982. Particle sampling and measurement. Environmental Science and Technology, 16:237-244.
Feitshans, I.L. 1991. Potential liability of Industrial Hygienists under the USA law. Part I: The dawning of the age of malpractice liability. National Asbestos Council Journal 9:31-35, 47-51.
Feldman, R.G. 1978. Urban lead mining: lead intoxication among deleaders. New England Journal of Medicine 298:1143-1145.
Findley, R.W. and D.A. Farber. 1988. Environmental Law. West Publishing Company. St. Paul, MN. 348 pp.
Fischbein, A., K.E. Anderson, S. Sassa, R. Lilis, S. Ken, S.L. Sarkozi and A. Kappas. 1981. Lead poisoning from do-it-yourself heat guns for removing lead-based paint: report of two cases. Environmental Research 24:425-431.
Fuller, F.H. and P.E. Smith. 1981. Evaluation of heat stress in a hot workshop by physiological measurements. American Journal of the Industrial Hygiene Association. 42:32-37.
Hammer, W. 1985. Occupational safety management and engineering. Prentice-Hall, Inc. Englewood, NJ. 511 pp.
Harber, P. 1984. Medical evaluations for respirator use. Journal of Occupational Medicine. 26:496-502.
Holak, W., 1975. Analysis of paints for lead by Atomic Absorption Spectrometry. Analytical Chemical Acta. 74:216-219.
Holmess, D.L. and J.R. Nethercott. 1988. Acute lead intoxication in a group of demolition workers. Applied Industrial Hygiene. 3:388-341.
Hood, J.B. and B.A. Hrdy. 1984. Workers' Compensation and Employees Protection Laws. West Publishing Company. St. Paul, MN. 274 pp.
Karam, H.S. and B.D. Beck. 1990. Current issues in determining acceptable lead concentrations in soil. Comments on Toxicology. 3:509-529.
Kendig, B. 1990. Environmental liability in real estate ownership. Mt. Hope Publishing Company. Rochester, NY. 104 pp.
Lange, J.H. 1991. A suggested air standard for lead to protect the public during lead abatement activities. Medical Hypotheses 36:211-212.
Lange, J.H. 1991a. Hazardous Waste Training: Meeting OSHA and practical requirements. Journal of the Pennsylvania Academy of Science. 65:145-152.
Lange, J.H. 1991b. Abatement contract specification documents: a discussion of issues. Journal of the Pennsylvania Academy of Science (accepted).
Lange, J.H., P.A. Rosato and G. Kaiser. 1991. Future directions of environmental assessments and audits. Journal of the Pennsylvania Academy of Science. 65:84-88.
Lange, J.H., P.A. Rosato and P.L. Spence. 1991a. A medical surveillance program for hazardous waste activities and asbestos abatement operation for a consulting engineering firm. Journal of Environmental Science and Health. A26:953-970.
Lange, J.H. and P.L. Spence. 1990. Practice, procedures and control during abatement of lead containing building materials. Journal of the Pennsylvania Academy of Science. 63:218-219 (abstract).
Lauren, G.R., Y.J. Kneip, R.E. Albert and F.S. Kent. 1971. X-Ray Fluorescence; detection of lead in wall paint. Science 172:466-486.
Leidel, N.A., K.A. Busch and J.R. Lynch, 1977. Occupational exposure sampling strategy manual. National Institute of Occupational Safety and Health (NIOSH) (DHEW Pub. nos. 77-173), Cincinnati, OH.
Lempert, B.L. 1984. Compendium of hearing protection devices. Sound and Vibration. 18:26-39.
Lioy, P.J. and M.J.Y. Lioy. 1983. Air sampling instruments for evaluation of atmospheric contaminants. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.
Lord, H.W., W.S. Gatley and H.A. Evensen. 1980. Noise Control for Engineers. McGraw-Hill Book Company, New York, NY. 435 pp.
Maryland Department of the Environment. 1983. Title 26, subtitle 02. Occupational, Industrial, and Residential Hazards, 26.02.07. Procedures for abating lead containing substances from buildings, Baltimore, MD.
Maryland Department of Licensing and Regulation, 1984. COMAR 09.12.32. Maryland Occupational Safety and Health Standard for Occupational Exposure to Lead: in Construction Work. Baltimore, MD.
Massachusetts Department of Labor and Industries. 1988. 454 CMR 22.00. Deleading regulations, Boston, MA.



Melius, J.M. 1986. Medical surveillance for hazardous waste workers. Journal of Occupational Medicine. 28:679-683.
Merritt, F.S. 1976. Standard Handbook for Civil Engineers. McGraw-Hill Book Company, New York, NY.
National Institute of Building Sciences. 1988. Lead-based paint testing, abatement, cleanup and disposal guidelines. Washington, DC. 237 pp.
National Institute of Occupational Safety and Health. 1986. Occupational exposure to hot environments. U.S. Department of Health and Human Services, Washington, DC. DHHS (NIOSH) Publication number 86-113.
NIOSH/OSHA/USCG/EPA. 1985. Occupational Safety and Health Guidance Manual for Hazardous Waste Site Activities. U.S. Department of Health and Human Services, Washington, DC.
Occupational Safety and Health Administration. 1989. Air contaminants: Final Rule (29 CFR 1910), Federal Register 54:2329-2984.
Occupational Safety and Health Administration. 1990. Toxic and Hazardous Substances. 29 Code of Federal Regulations 1910.1000. Washington, D.C.
Occupational Safety and Health Administration. 1990a. Lead. 29 Code of Federal Regulations 1910.1025. Washington, D.C.
Office of Technology Assessment. 1985. Reproductive Health Hazards in the Workplace. Washington, DC. (Government Printing Office, OTA-BA-266).
Olishifski, J.B. 1987. Fundamentals of Industrial Hygiene. National Safety Council, Chicago, IL. 753 pp.
Pennsylvania Department of Environmental Resources. 1988. Title 25. Environmental Resources. Harrisburg, PA.
Pennsylvania Department of Environmental Resources. 1988a. Hazardous Waste Cleanup Act (Act 1988-108; House Bill 1852). Harrisburg, PA.
Pettit, T. and H. Linn. 1987. A guide to safety in confined spaces. U.S. Department of Health and Human Services. DHHS (NIOSH) publication number 87-113.
Plummer, R.W., T.J. Stobbe, J.E. Morgensen and L.K. Jeram. 1987. Minimizing employee exposure to toxic chemical releases. Noyes Data Corporation, Park Ridge, NJ.
Que Hee, S.S., B. Peace, C.S. Clark, J.R. Boyle, R.L. Bornschein and P.B. Hammond. 1985. Evolution of efficient methods to sample lead sources, such as house dust and hand dust, in homes of children. Environmental Research. 38:77-95.
Rajhans, G.S. and D.S.L. Blackwell. 1985. Practical guide to respirator usage in Industry. Butterworth Publishers, Boston, MA. 161 pp.
Rekus, J.F. 1988. The occupational lead hazard. pp. 1-13. In: Proceeding of the Steel Structures Painting Council: Lead paint removal. (Appleman, B.R., L.M. Smith and J.A. Zappa, eds.) Steel Structures Painting Council, Pittsburgh, PA. 209 pp.
Rey-Alvarez, S. and T. Menke-Hargrave. 1987. Deleading dilemma: pitfalls in the management of childhood lead poisoning. Pediatrics 79:214-217.
Rieke, F.E. 1969. Lead intoxication in shipbuilding and shipscrapping 1941 to 1968. Archives of Environmental Health. 19:521-539.
Rom, W. 1983. Environmental and Occupational Medicine. Little, Brown and Company, Boston, MA. 1015 pp.
Rork, R., M.K. White and H. Linn. 1984. Personal protective equipment for hazardous waste material incidents: selection guide. US Department of Health and Human Services, Division of Safety Research, Morgantown, WV.
Rosenstock, L. and M.R. Cullen. 1986. Clinical Occupational Medicine. W.B. Saunders, Philadelphia, PA. 305 pp.
Sawyer, C.J. 1985. Contingency plans. pp. 327-348. In: Protecting personnel at Hazardous Waste Sites. (S.P. Levine and W.F. Martin, eds.) Butterworth Publishers, Boston, MA. 366 pp.
Saurai, H., M. Sagita and K. Tsuchiya. 1974. Biological response and subjective symptoms in low level lead exposure. Archives of Environmental Health 29:157-163.
Sayer, J.W. and D.W. Wilson. 1970. A spot test for detection of lead in paint. Pediatrics. 46:783-785.
Schaber, G.D. and C.D. Rohwer. 1984. Contracts. West Publishing Company, St. Paul, MN. 425 pp.
Schwartz, J., P.J. Landrigan, E.L. Baker, W.A. Orenstein and I.H. Von Lindern. 1990. Lead-induced anemia: dose-response relationships and evidence for a threshold. American Journal of Public Health. 80:165-168.
Schwope, A.D., P.P. Costas, J.O. Jackson, J.O. Stull and D.L. Weitzman. 1987. Guidelines for the selection of chemical protective clothing. American Conference of Governmental Industrial Hygienists, Inc. Volume I and II. Cincinnati, OH.
Secchi, G.C. and L. Alessio. 1974. Laboratory results of some biological measures in workers exposed to lead. Archieves of Environmental Health. 29:351-354.
Sheldon, L., M. Umana, J. Bursey, W. Gutlenecht, R. Handy, P. Hyldburg, L. Michael, A. Moseley, J. Raymer, D. Smith, C. Sparacino and M. Warner. 1986. Biological monitoring techniques for human exposure to industrial chemicals. Noyes Publications, Park Ridge, NJ.
Solomon, R.L. and J.W. Hartford. 1976. Lead and cadmium in dust and soil in a small urban community. Environmental Science and Technology. 10:773-777.
Spangenberg, W.C. and H. Stower. 1988. History of lead containing coatings and pigments. pp. 6-9. In: Lead paint removal from structures: Proceedings of the Lead Paint Removal Conferences (B.R. Appleman and J. Rex, eds.) Steel Sructures Painting Council, Pittsburgh, PA. 114 pp.
Steffen, R.T. 1977. Agency-Partnership. West Publishing Company, St. Paul, MN. 364 pp.
Specdata. 1988. Drumond Chemicals, Inc. New York, NY
Subramanian, K.S., J.W. Conner and J.C. Meranger. 1991. Leaching of antimony, cadmium, copper, lead, silver, tin and zinc from copper piping with non-lead-based soldered joints. Journal of Environmental Science and Health. A 26:911-929.
Ter, Haar, G., and R. Aronow. 1974. New information on lead in dirt and dust as related to the childhood lead problem. Environmental Health Perspectives. 7:83-89.

Tinklenberg, G. 1988. EP Tox: Michigan's experience. pp. 175-209. In: Proceeding of the steel structures painting council: lead paint removal (Appleman, B.R., L.M. Smith and J.A. Zappa, eds.) Steel Structures Painting Council, Pittsburgh, PA 209 pp.

Tomokani, K. and M. Ogata. 1976. Relationship between lead concentration in blood and biological response to porphyrin metabolism in workers occupationally exposed to lead. Archives of Toxicology 35:238-246.

U.S. Congress, Office of Technical Assessment. 1986. Transportation of Hazardous Materials, OTA-SET-304, Washington, DC.

U.S. Department of the Interior. 1987. Construction Safety Standards. Division of Safety, Engineering and Research. Denver, CO. 510 pp.

U.S. Department of Labor. 1981. Hazard Communications: Final rule (29 CFR 1910.1200). Federal Register 52:31852-31882.

West, W.H. 1986. Hazardous Waste Management-an industrial perspective. In: Management of Toxic and Hazardous Wastes. (Bhatt, H.G., R.M. Sykes, and T.L. Sweeney, eds.) Lewis Publishers, Inc., Chelsa, MI. 418 pp.

Zenz, C. 1988. Occupational Medicine. Yearbook Medical Publishers, Inc. Chicago, IL. 1273 pp.

Zimmer, F.E. 1961. Lead posioning in scrap-metal workers. Journal of the American Medical Association. 175: 238-240.



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