

MONOCHLORAMINE SOLUTION

Monochloramine was prepared by mixing ammonium chloride with sodium hypochlorite in a 2,5:1 molar ratio. Stock monochloramine at 500-600 mg/l was prepared by dissolving 1,15 g of ammonium chloride in 900 ml of distilled water, adjusting the pH to 8,5-9,0 with sodium hydroxide and then slowly adding 6 ml of sodium hypochlorite (10% w/v Cl₂; 600 mg Cl₂). The final volume was made up to 1 l. Stock monochloramine was stored in the dark at 4°C.

MONOCHLORAMINE DETERMINATIONS

Monochloramine concentrations were determined by titration with ferrous ammonium sulphate with *N,N*-diethyl-*p*-phenylenediamine as a colorimetric indicator (Anon. 1985).

REACTION VESSEL

A simple plumbing system was used as a reaction vessel (Fig. 1). Several components of this system were derived from the more complex model described by Muraca *et al.* (1987). The system included a 20 l glass reservoir, a 2 l glass jar containing a mixture of rubber washers to a depth of 40 mm, 7,5 m of 9 mm diam. copper tubing, 4 m of 10 mm diam. silicone tubing and

a variable speed peristaltic pump. The two glass containers were held in a water bath.

DISINFECTION EXPERIMENTS

Before each experiment the plumbing system was flushed and then filled with 18 l of hot (80-90°C) autoclaved tap water at pH between 8,4 and 8,6. The water was allowed to cool to 30°C and then inoculated with either *L. pneumophila* or *E. coli*. *Legionella pneumophila* was grown on slopes of BCYE agar which were incubated for 72 h at 37°C. Bacterial suspensions were prepared by washing three slopes with sterile tap water. This provided an initial concentration of 3-4 x 10⁵ cells/ml. The *L. pneumophila* was then allowed to circulate in the plumbing system at a flow rate of about 1 l/min for 2 h. Immediately before adding a predetermined volume of stock monochloramine to the 20 l container, a 1 ml sample was collected and the flow rate was increased to 3-4 l/min to facilitate mixing of the disinfectant.

After the addition of monochloramine, further 1 ml samples were taken at 2 min intervals for 20 min and then at 25, 30 and 40 min. Each sample was serially diluted in sterile tap water. Each dilution bottle contained 1 ml of 10% (w/v) sodium thiosulphate, to neutralize monochloramine, and 0,1 ml volumes of each dilution were plated on BCYE agar in triplicate. The plates were incubated at 37°C in air containing

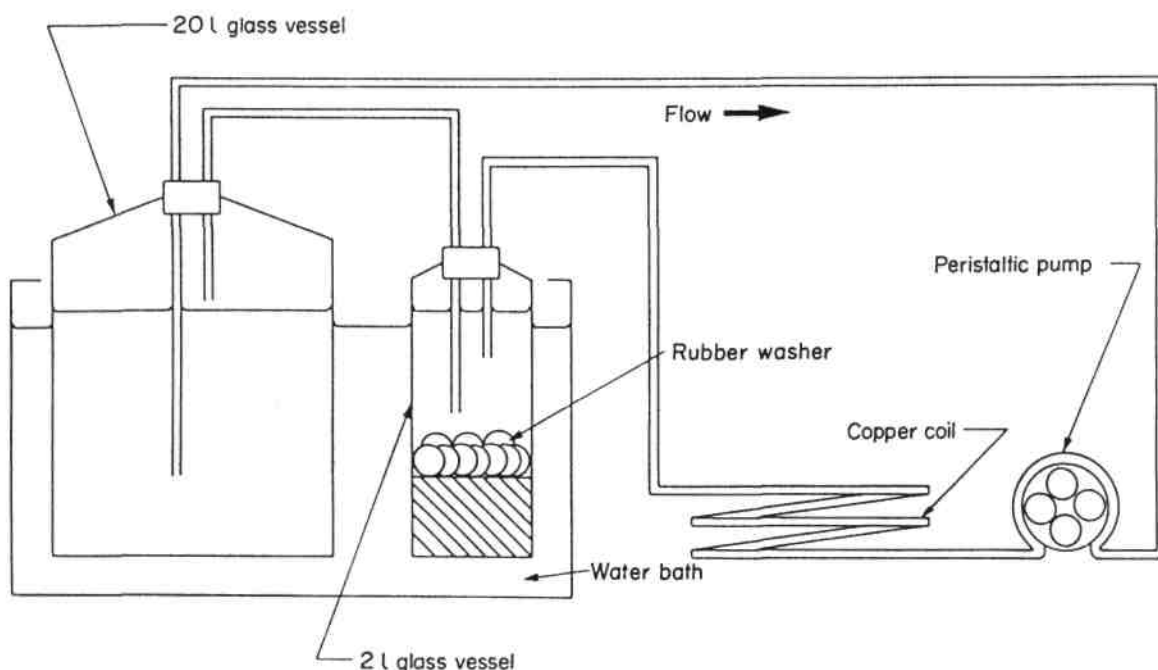


Fig. 1. Schematic diagram of the model plumbing system used in the disinfection experiments.

3% CO₂. Colonies of *L. pneumophila* were counted after incubation for 72 h. Monochloramine concentrations were determined after 2 and 40 min. The loss of monochloramine never exceeded 0,1 mg/l.

Experiments with *E. coli* were similar but the organisms were harvested by centrifugation (7000 g for 15 min) of 10 ml of an overnight Tryptone Soya Broth (Oxoid) culture, incubated at 35°C for 18 h. The bacteria were resuspended in 10 ml of sterile tap water, inoculated into the plumbing system and allowed to circulate for 30 min before the addition of monochloramine. After the addition of monochloramine, 1 ml samples were collected at 2 min intervals for 20 min and then at 5 min intervals for a further 40 min. The initial concentration of *E. coli* in the system was 3-4 x 10⁵ cells/ml. Colonies of *E. coli* were counted after incubation at 35°C for 24 h.

DETERMINATION OF 99% INACTIVATION TIMES

For each disinfection experiment, the time taken to inactivate 99% of the original inoculum was determined by plotting log percentage survival against time (min). After initial lag periods, these plots produced straight lines with correlation coefficients (r^2), as determined by regression analysis, in the range 0,96-0,99.

STABILITY OF MONOCHLORAMINE

Samples of potable water containing monochloramine were collected from public supplies and were incubated in closed flasks at either 30°C or 55°C. Monochloramine concentrations were monitored throughout the course of the incubation.

Results

The ability of *L. pneumophila* to survive in the model system in the absence of monochloramine is shown in Fig. 2. There is no significant reduction in the numbers of viable organisms over a 6 day period.

The results of the disinfection experiments show that *L. pneumophila* is more sensitive than *E. coli* to inactivation by monochloramine (Fig. 3). The results are expressed in terms of the formula of Watson (1908) in which $k = C^n \times t$ where k is a constant, C is the concentration of disinfectant, t is the time taken to inactivate 99% (in this case) of the original inoculum and n , the slope of the lines in Fig. 3, is the coefficient of dilution. Comparisons of $C \times t$ products for different organisms require n to approximate 10 (Rubin *et al.* 1983; Wickramanayake *et al.* 1984). The values of n from Fig. 3 were 0,98 for *L. pneumophila* and 0,97 for *E. coli*. The average $C \times t_{99}$ for *L. pneumophila*

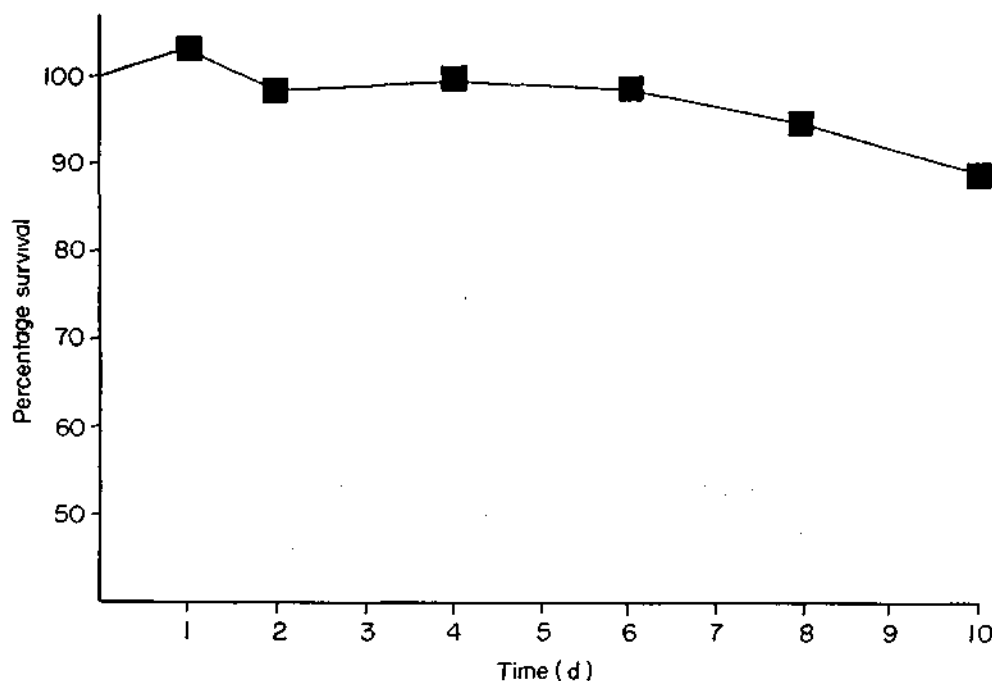


Fig. 2. Survival of *Legionella pneumophila* in the model system. The system was prepared, inoculated with *L. pneumophila* and sampled as described for the disinfection experiments. Each point represents the average of triplicate analyses.

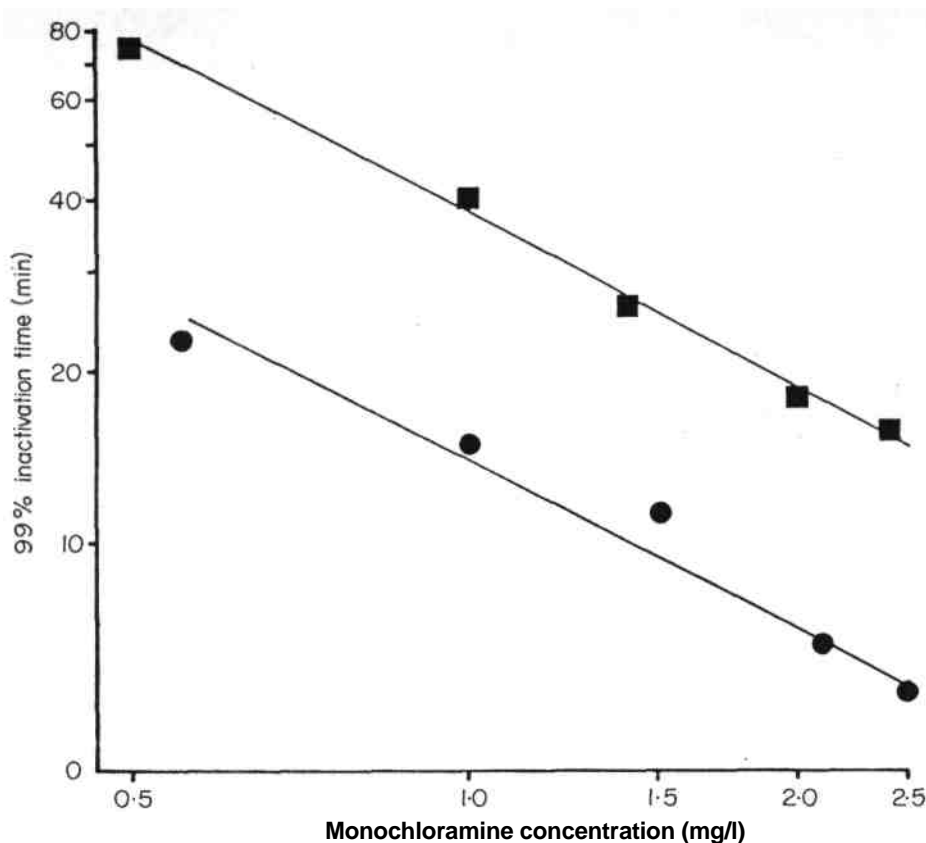


Fig. 3. Inactivation of ●, *Legionella pneumophila* and ■, *Escherichia coli* by monochloramine. The concentrations of monochloramine were those measured 2 min after the addition of monochloramine to the model system. Lines of best fit were determined using regression analysis.

was 15 mg.min/l while for *E. coli* it was 37 mg.min/l.

In the disinfection experiments with *L. pneumophila* the bacteria were inoculated into the model system 2 h before the addition of monochloramine. Extending the time between inoculation and addition of monochloramine to up to 6 d did not increase the resistance of *L. pneumophila* to inactivation by monochloramine (results not shown).

Colbourne *et al.* (1988) reported that heat shock can lead to the recovery of previously 'viable non-culturable' *L. pneumophila* from water samples. This technique was tried but heat shock did not improve the recoveries of *L. pneumophila* during disinfection experiments.

The decay of monochloramine in potable water was measured at 30°C and 55°C. Before incubation the water contained 1.3 mg/l monochloramine. As expected, the decay was more rapid at 55°C but even after 50 h the water still contained 0.35 mg/l (Fig. 4).

After 5 d at 30°C the water contained 0.8 mg/l (Fig. 4).

Discussion

Experimental conditions were chosen both to favour the survival of *L. pneumophila* and to be consistent with those found in local chloraminated supplies. This was achieved by a simple plumbing system and tap water at 30°C and pH 8.4-8.6. In South Australia, chloramination has generally been introduced for supplies incorporating long above-ground pipelines and, in summer, water temperatures are typically in the range 25-30°C. Where possible relatively high pH levels are maintained in these supplies to promote the stability of monochloramine.

The disinfection experiments showed that *L. pneumophila* was more sensitive to monochloramine than the faecal indicator *E. coli*. The average $C \times t_{99}$ for *L. pneumophila* was 15 mg.min/l compared with 37 mg.min/l for *E. coli*. In comparison it has been found that *L. pneumophila* is less sensitive than *E. coli* to disinfection by chlorine (Kuchta *et al.* 1983).

There are two advantages in using monochloramine as a biocide for *L. pneumophila* in

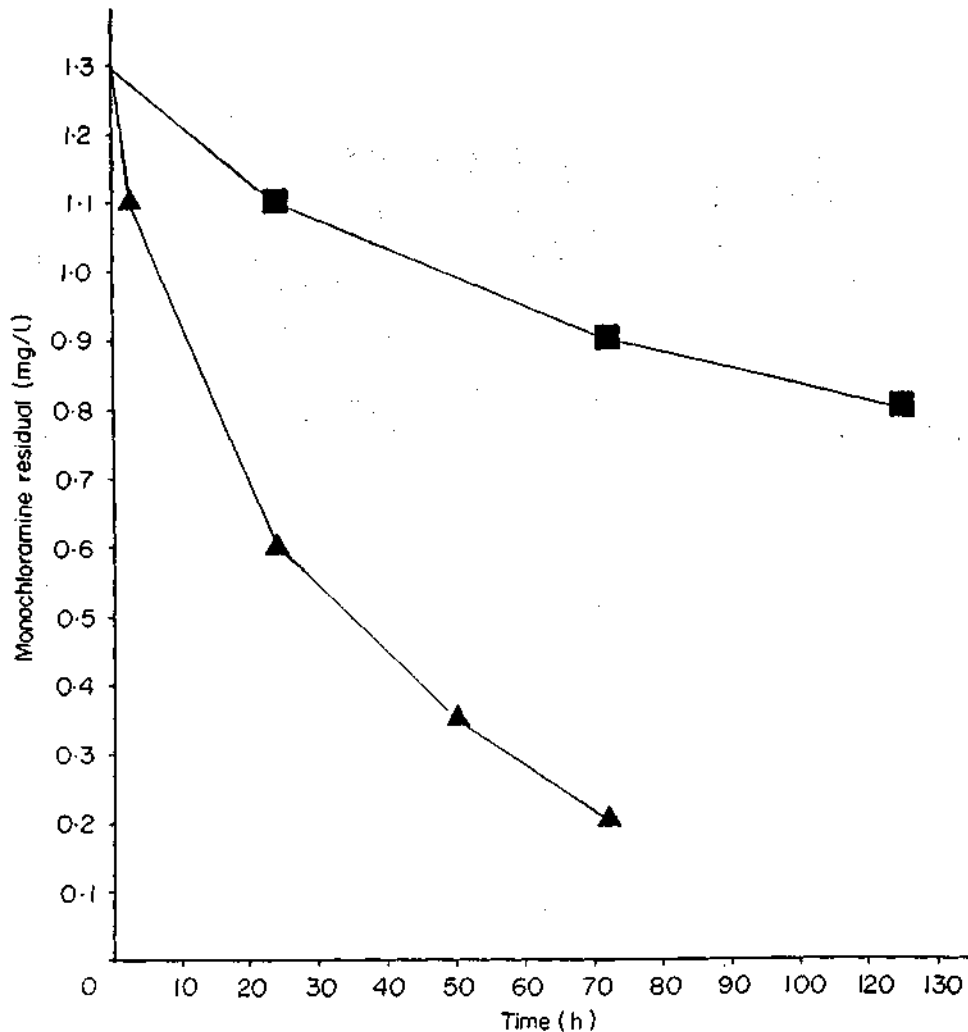


Fig. 4. Decay of monochloramine in potable water incubated at ■, 30°C and ▲, 55°C. The water used was collected from a chloraminated public supply. Each point represents the average of triplicate analysers.

plumbing systems: it is relatively stable; and it can penetrate biofilms (LeChevallier *et al.* 1988). *Legionella pneumophila* has been found in water and sediments from hot water systems (Fisher-Hoch *et al.* 1982; Wadowsky *et al.* 1982; Stout *et al.* 1985) and from the surfaces of pipes and plumbing fittings (Ciesielski *et al.* 1984; Colbourne *et al.* 1984; Voss *et al.* 1986). The colonization of hot water systems by *L. pneumophila* has been a particular problem in hospitals (Fisher-Hoch *et al.* 1982; Wadowsky *et al.* 1982; Stout *et al.* 1985). Control of *L. pneumophila* in these systems requires the maintenance of temperatures 60°C or greater in both the storage tank and distribution mains (Groothuis *et al.* 1985; Stout *et al.* 1986). However, hospital water systems are often kept below this temperature to reduce the risk of scalding. Even at elevated temperatures monochloramine is relatively stable. Incubation of chloraminated water

at 55°C resulted in only a gradual loss of disinfectant; after 50 h 0.35 mg/l remained in samples that originally contained 1.3 mg/l monochloramine. The greater stability of monochloramine at lower temperatures combined with its ability to penetrate biofilms should control the levels of *L. pneumophila* in cooler parts of plumbing systems.

In laboratory experiments alternative disinfectants such as chlorine, heat, ozone and u.v. light have been found to be effective against *L. pneumophila* (Muraca *et al.* 1987). In practice, however, each has limitations when used to provide continual protection throughout a water distribution system (Muraca *et al.* 1988). Heat eradication is generally restricted to use as a shock treatment, u.v. light and ozone do not offer any residual protection and chlorine decays rapidly.

Extrapolating results from laboratory experi-

ments to field situations can be difficult. For example, it has been reported that agar-passaged *L. pneumophila* is more sensitive to chlorine than non-passaged *L. pneumophila* (Kuchta *et al.* 1985). Conversely, it has been observed that laboratory results underestimate the effectiveness of monochloramine in field situations (Wolfe & Olson 1985). On balance, however, it is concluded that the presence of monochloramine in water should contribute to the prevention of growth of *L. pneumophila* in plumbing systems in large buildings. In addition, monochloramine may be useful as a biocide for *L. pneumophila* in water distribution systems and cooling towers.

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References

- ANON. 1985 *Standard Methods for the Examination of Water and Wastewater* 16th edn, pp. 306-309. Washington: American Public Health Association.
- BAIRD, I.M., PORRS, W., SMILEY, J., GLICK, N., SCHLEICH, S., CONNOLE, C. & DAVISON, K. 1983 Control of endemic nosocomial legionellosis by hyperchlorination of potable water. In *Legionella: Proceedings of the Second International Symposium* ed. Thornsberry G, Balows, A., Feeley, J.C. & Jakubowski, W. p. 333. Washington: American Society for Microbiology.
- BARRETT, S.E., DAVIS, M.K. & MCGUIRE, M.J. 1985 Blending chloraminated and chlorinated waters. *Journal of the American Water Works Association* 77, 50-61.
- BARTLETT, C.L.R. 1983 Potable water as reservoir and means of transmission. In *Legionella: Proceedings of the Second International Symposium* ed. Thornsberry, C, Balows, A., Feeley, J.C. & Jakubowski, W. pp. 210-215. Washington: American Society for Microbiology.
- BARTLETT, C.L.R., SWANN, R.A., CASAL, J., CANADA ROYO, L. & TAYLOR, A.G. 1983 Recurrent Legionnaires disease from a hotel water system. In *Legionella: Proceedings of the Second International Symposium* ed. Thornsberry, C, Balows, A., Feeley, J.C. & Jakubowski, W. pp. 237-239. Washington: American Society for Microbiology.
- BEST, M., YU, V.L., STOUT, J., GOETZ, A., MUDER, R.R. & TAYLOR, F. 1983 Legionellaceae in the hospital water supply epidemiological link with disease and evaluation of a method for control of nosocomial Legionnaires disease and Pittsburgh pneumonia. *Lancet* ii, 307-310.
- CARTER, R.F. 1970 Description of a *Naegleria* isolated from two cases of primary amebic encephalitis, and of the experimental pathological changes induced by it. *Journal of Pathology* 100, 217-244.
- CIESIELSKI, C.A., BLASER, M.J. & WANG, W.L. 1984 Role of stagnation and obstruction of water flow in isolation of *Legionella pneumophila* from hospital plumbing. *Applied and Environmental Microbiology* 48, 984-987.
- COLBOURNE, J.S., PRATT, D.J., SMITH, M.G., FISHER-HOCH, S.P. & HARPER, D. 1984 Water fittings as sources of *Legionella pneumophila* in a hospital plumbing system. *Lancet* i, 210-213.
- COLBOURNE, J.S., DENNIS, P.J., TREW, R.M., BERRY, C. & VESEY, G. 1988 *Legionella* and public water supplies. *Water Science and Technology* 20 (11/12), 5-10.
- FISHER-HOCH, S.P., SMITH, M.G. & COLBOURNE, J.S. 1982 *Legionella pneumophila* in hospital hot water cylinders. *Lancet* i, 1073.
- FRASER, D.W. 1985 Potable water as a source for legionellosis. *Environmental Health Perspectives* 62, 337-341.
- GROOTHUIS, D.G., VEENENDAAL, H.R. & DIJKSTRA, H.L. 1985 Influence of temperature on the number of *Legionella pneumophila* in hot water systems. *Journal of Applied Bacteriology* 59, 529-536.
- KUCHTA, J.M., STATES, S.J., MCNAMARA, A.M., WADOWSKY, R.M. & YEE, R.B. 1983 Susceptibility of *Legionella pneumophila* to chlorine in tap water. *Applied and Environmental Microbiology* 46, 1134-1139.
- KUCHTA, J.M., STATES, S.J., MCLAUGHLIN, J.E., OVERMEYER, J.E., WADOWSKY, R.M., MCNAMARA, A.M., WOLFORD, R.S. & YEE, R.B. 1985. Enhanced chlorine resistance of tap water—adapted *Legionella pneumophila* as compared with agar medium-passaged strains. *Applied and Environmental Microbiology* 50, 21-26.
- LECHEVALLIER, M.W., CAWTHON, CD. & LEE, R.G. 1988 Inactivation of biofilm bacteria. *Applied and Environmental Microbiology* 54, 2492-2499.
- MASSANARI, R.M., HELMS, C, ZEITLER, R., STREED, S., GILCHRIST, M., HALL, N., HAUSLER JR., W., JOHNSON, W., WINTERMEYER, L., MUHS, J.S. & HIERHOLZER JR., W.J. 1983 Continuous hyperchlorination of a potable water system for control of nosocomial *Legionella pneumophila* infections. In *Legionella: Proceedings of the Second International Symposium* ed. Thornsberry, C, Balows, A., Feeley, J.C. & Jakubowski, W. pp. 334-336. Washington: American Society for Microbiology.
- MURACA, P., STOUT, J.E. & Yu, V.L. 1987 Comparative assessment of chlorine, heat, ozone and UV light for killing *Legionella pneumophila* within a model plumbing system. *Applied and Environmental Microbiology* 53, 447-453.
- MURACA, P.W., Yu, V.L. & STOUT, J.E. 1988 Environmental aspects of Legionnaires disease. *Journal of the American Water Works Association* 80, 78-86.
- RUBIN, A.J., ENGEL, J.P. & SPROUL, O.J. 1983 Disin-