Final Report: NOAA Puerto Rico Pharmaceutical Wastes,
C. Van Baalen, January 11, 1980.

Individual pharmaceutical wastes from operations in Puerto Rico were examined for toxicity towards representative types of microalgae. Three algal test species: the blue-green alga, Agmenellum quadruplicatum, strain PR-6 (our isolate); the green alga, Chlorella autotrophica, strain 580 (obtained from R.R.L. Guillard); and the diatom, Cylindrotheca sp. strain N-1 (our isolate) were grown on medium ASP-2 (Provosoli, et al., 1957; Van Baalen, 1962) at 30°C, see Figure 1 for details. The wastes supplied to us were upon receipt dispensed into clean screw cap glass tubes and frozen until tested. The Schering sample was received January 3, 1980 and will be tested shortly. The untreated wastes were added directly to the growth medium, v/v, just before inoculation and incubation.

Growth rates and lag times in initiation of growth were the experimental endpoints. A representative set of growth curves is given in Figure 2. From such data the generation times recorded in Tables 1 and 2 were calculated. Lag times greater than one generation time are significant. Lags could represent a lag in growth by all cells in the population or a fractional kill of the inoculum. The turbidimetric measure of growth as used here will not distinguish between these two alternatives.

The results with the blue-green alga, PR-6; the diatom N-1; and the green alga, 580; demonstrate the highly toxic nature of the Bristol sample. The Upjohn sample was only inhibitory to

PR-6 (Table 1). It should be noted that in algal assays such as these that the cell concentrations (in terms of chlorophyll a) are roughly 1000 times that of natural populations. Hence in nature the ratio toxicant/cells will be higher and samples like Bristol or Upjohn could be effective at considerably lower concentrations.

The Upjohn sample gave the same results with organism PR-6 whether it was filtered or not filtered (0.4 μm Nucleopore polycarbonate type filter). The biphasic nature of the Bristol sample precluded filtering it. The Bristol sample was examined further using organism PR-6. It showed evident toxicity as low as 25 ppm, and at 50 ppm was completely inhibitory to the inoculum (Table 2 . The nature of the toxic material(s) in the Bristol sample are unknown. Preliminary experiments on the effect of exposure to sunlight on the toxicity of Bristol sample were conducted in quartz tubes containing 250 ppm Bristol sample in filtered sea water. It appears that there is a lessening of toxicity after 24 hours followed by an increase in toxicity upon further exposure. This rather curious effect of sunlight on the Bristol sample needs to be confirmed.

In summary, of the six pharmaceutical wastes tested, four (Pfizer, Capri, Squibb, Merck) were not toxic as judged by their effect on growth rate of three algal types. The Upjohn sample showed what has come to be a common observation, selective toxicity towards one kind of alga (Winters et al., 1977; Batterton et al., 1978). The Bristol sample was not only toxic at rather low levels but was also toxic to all three test species. Presumably

this indicates that the toxic material in it is a general metabolic poison active in both prokaryotic and eucaryotic organisms.

References

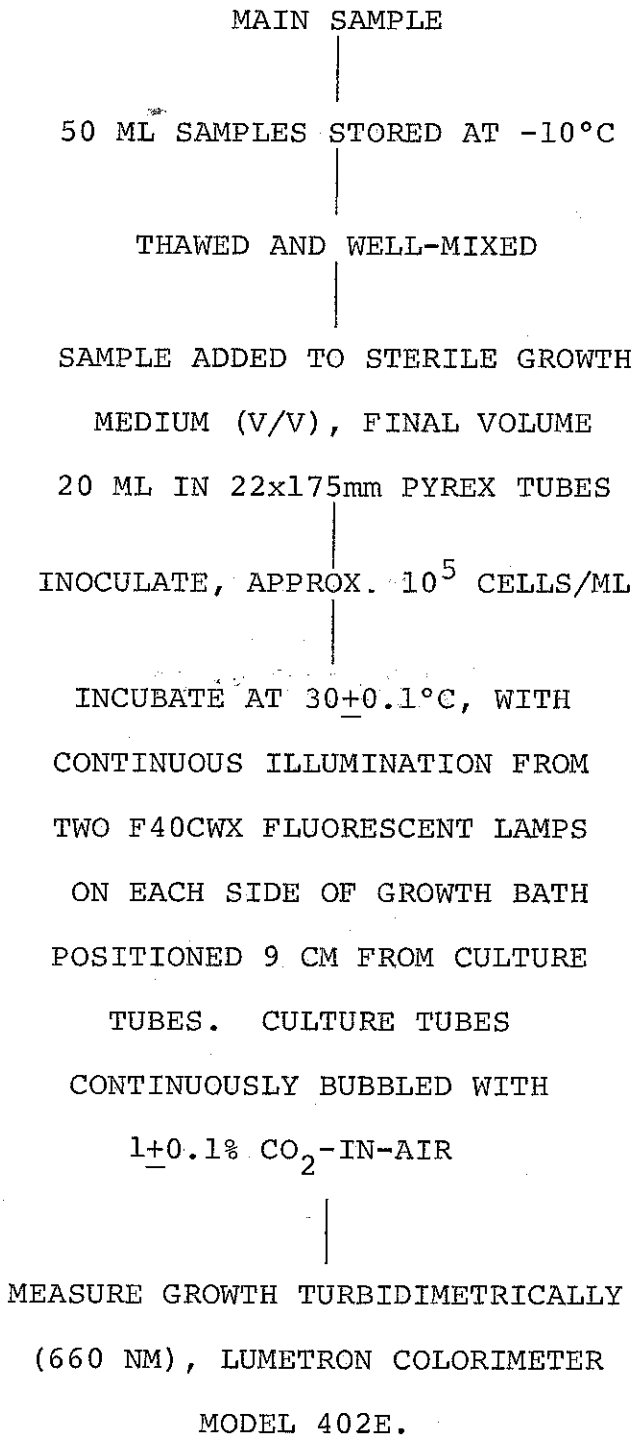
Batterton, J. C., K. Winters and C. Van Baalen. 1978. Anilines: selective toxicity to blue-green algae. Science 199, 1068-1070.

Provasoli, L., J. J. A. McLaughlin and M. R. Droop. 1957. The development of artificial media for marine algae. Arch. Mikrobiol. 25, 392-428.

Van Baalen, C. 1962. Studies on marine blue-green algae. Bot. mar. 4, 129-139.

Winters, K., J. C. Batterton and C. Van Baalen. 1977. Phenalen-1-one: occurrence in a fuel oil and toxicity to microalgae. Environ. Sci. Technol. 11, 270-272.

Figure 1. Protocol for testing waste material samples



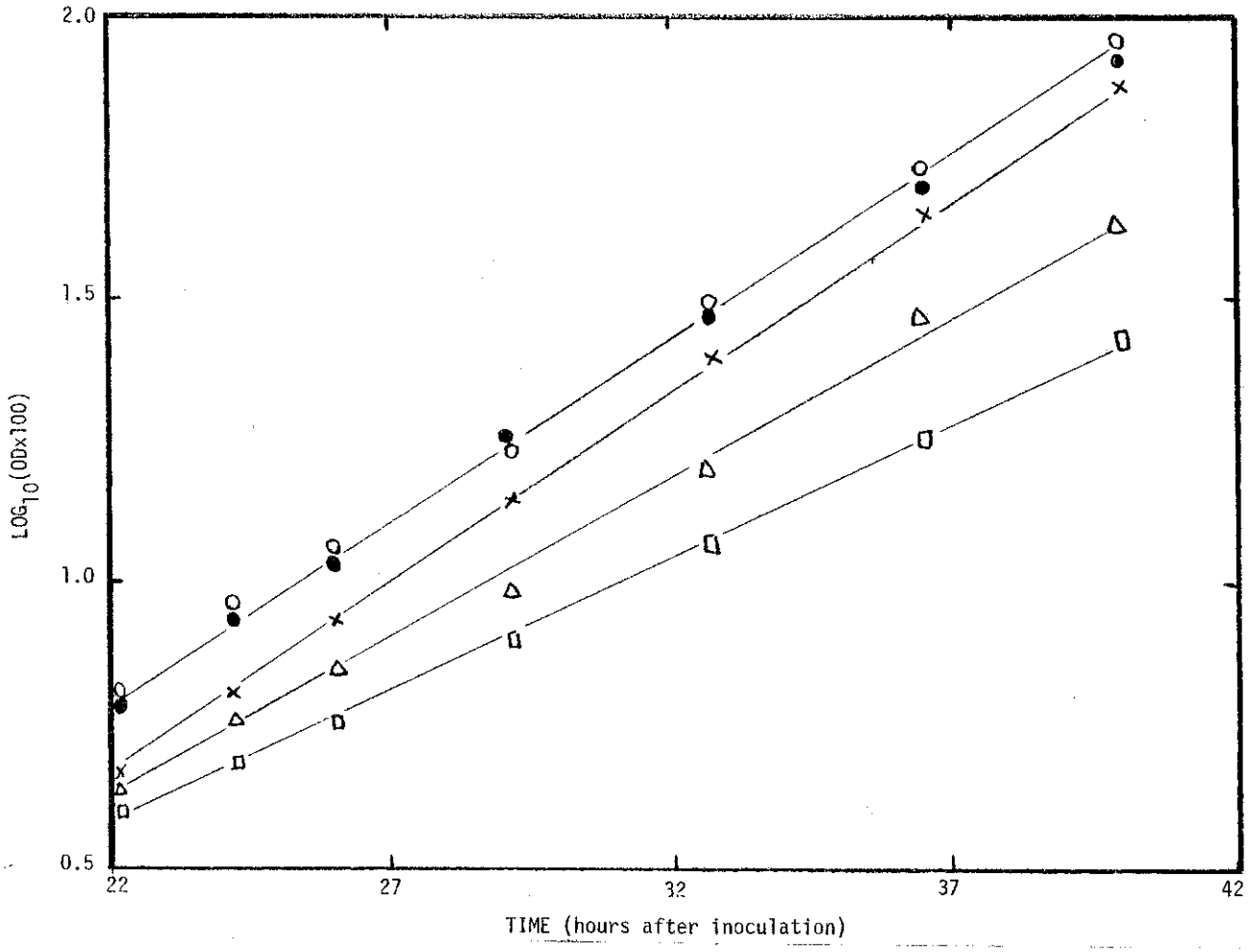


Figure 2. Growth of organism PR-6 in the presence of Squibb waste; O and ● are controls, X plus 250ppm waste, Δ 2500ppm, □ 5000ppm. OD (optical density) is proportional to cell number to OD = 1.0, at OD = 1.0 for organism PR-6 the cell dry weight is 0.25 mg ml^{-1}.

Table 1. Algal growth rates, as generations per day, in the presence of pharmaceutical wastes.

Waste Material Identification	Concentration ppm	Organisms PR-6	N-1	580
Controls	0	5.3±.3	4.7±.3	2.9±.3
Bristol	250	NG[a]	2.4	NG
	500	NG	NG	NG
	2500	NG	NG	NG
	5000	NG	NG	NG
Upjohn	250	NG	4.5	3.0
	500	NG	4.5	3.0
	2500	NG	4.0	3.0
	5000	NG	2.5(26)[b]	3.1
Merck	250	4.9	4.7	3.0
	500	4.9	4.5	3.0
	2500	4.3	4.6	2.6
	5000	2.5	4.6	2.6
Squibb	250	5.3	4.5	2.9
	500	5.3	4.5	2.9
	2500	4.5	3.5(17)	3.1
	5000	4.1	3.5(24)	3.1
Capri	250	5.4	4.7	2.9
	500	5.4	4.7	2.9
	2500	5.4	4.6	2.8
	5000	5.0	4.6	2.8
Pfizer	250	5.2	4.7	2.8
	500	5.1	4.6	2.8
	2500	5.1	4.7	2.8
	5000	5.1	4.5	2.8

[a] NG means no growth for up to 5 days incubation.

[b] Number in parenthesis is lag time in hours as compared to a control.

Table 2. The effect of Bristol Waste on growth rate of organism PR-6.

Concentration Bristol Waste ppm	Generations Per Day
0 (control)	5.0±.3
7	4.8
14	4.4
25	4.4 (72)[b]
50	NG[a]
125	NG
250	NG

[a] NG means no growth for up to 5 days incubation.

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	2500	2.3	4.6	2.8
	5000	1.1	4.7	2.8

[a] NG means no growth for up to 5 days incubation.

[b] Number in parenthesis is lag time in hours as compared to a control.

~~1979 Samples~~

Table 16. Algal growth rates, as generations per day, in the presence of pharmaceutical wastes. (1979 SAMPLES) GROWTH IN OPEN TEST-TUBES WITH CONTINUOUS AERATION WITH 1% CO_2-IN-AIR.

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	5000	5.1	4.5	2.8

[a] NG means no growth for up to 5 days incubation.

[b] Number in parenthesis is lag time in hours as compared to a control.