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Mutagenicity Produced by Aqueous Chlorination of Organic Compounds

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The filtrate from aqueous chlorination of unbleached kraft pulp has been shown by ANDER et al. (1977) to be mutagenic by the Ames test (McCANN et al. 1975) and confirmed by us. In a second paper by ERIKSSON et al. (1979) it was shown that substitution of chlorine dioxide for equivalent chlorine almost eliminated the mutagenicity, which we have also confirmed.

We have found that chlorination of groundwood also produces mutagenicity, and so does purified lignin separated from wood, showing that lignin and not carbohydrates or extractives is the main source of the mutagenicity of chlorinated pulp.

On analysing the chlorinated pulp filtrate by gas chromatography, very many peaks were found. Among the compounds so far identified and tested in pure form, such as chlorinated phenols, catechols and guaiacols, none has been found to be mutagenic by the Ames test.

METHODS

To simplify the task of analysis and identification and to make reproducible material available for study, pure compounds typical of structures found in the lignin molecule, in dilute aqueous solution of a concentration similar to that which might be expected from the lignin in pulp (0.004 - 0.008 molar), were treated with increasing equivalents of chlorine and chlorine dioxide per mole. A single Ames test using a constant volume (0.4 ml) of each solution was carried out (rather than duplicates). Since the resulting number of mutants was plotted against equivalents of chlorine applied per mole, any experimental deviation would show up on the curve.

RESULTS AND DISCUSSION

The first compound tested, acetovanillone, almost duplicated the mutagenicity found with similar treatment of pulp (Figure 1) (BUTSKY 1978). Subsequently it was found that virtually all lignin model compounds and all phenols tested, regardless of the nature of substituted side chains, produced particular patterns of mutagenicity versus equivalents of chlorine applied per mole of substrate, as exemplified in Figure 2. The number of revertant colonies produced often varied inversely with the toxicity. The peaks in the curves represent high mutagenicity accompanied by low toxicity, and the valleys represent either low mutagenicity or

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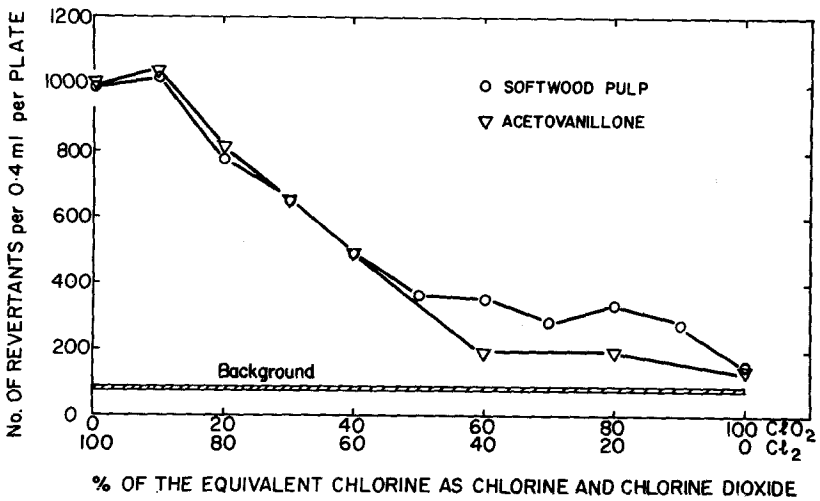

Figure 1. TA100 Ames revertants per plate for 0.4 ml of filtrate from treatment of unbleached kraft pulp with 6.8% equivalent chlorine as mixtures of chlorine and chlorine dioxide from 0 to 100% replacement compared with 0.4 ml of 8 millimolar acetovanillone treated with 7 equivalents per mole of mixtures of chlorine with 0 to 100% replacement by equivalent chlorine dioxide (1 g of chlorine dioxide is equivalent to 2.63 g of chlorine).

high toxicity, or both.

Toxicity toward the Ames Salmonella TA100 strain used was roughly estimated by visual comparison of the background lawn on the plate under a microscope, with 100% representing the same number of bacteria per unit area as the blank with no test solution (no toxicity) and 0 representing death of all bacteria. In most cases there is a good correlation between low toxicity and high mutagenicity, and vice versa.

All pure substances used in the chlorination experiments, many substances found to be products of reaction by chromatographic analysis, and many substances speculated to be possible reaction products were tested for mutagenicity (Table 1). None of the compounds in the chlorination experiments was found to be mutagenic before chlorine treatment. No chlorinated phenol was found positive in the Ames test. Among the 93 substances tested, only 1,3-dichloroacetone, 1,1,3,3-tetrachloroacetone, 3,6-dichloro-2-hydroxybenzaldehyde, dibromomethane and mucochloric acid were mutagenic and not toxic. Ames assays of hexachloroacetone and o-benzoquinone were sometimes positive and sometimes negative. Of these only 1,3-dichloroacetone has been identified in chlorination filtrate (STROMBERG and DE SOUSA 1979).

TABLE 1

Mutagenicity (±) of compounds assayed on TA100

phenol -
o-chlorophenol -
m-chlorophenol -
p-chlorophenol -
2,3-dichlorophenol -
2,4-dichlorophenol -
2,5-dichlorophenol -
2,6-dichlorophenol -
3,4-dichlorophenol -
3,5-dichlorophenol -
2,3,4-trichlorophenol -
2,3,5-trichlorophenol -
2,4,6-trichlorophenol -
2,6-dibromophenol -
guaiacol -
2,4-dichloroguaiacol -
tetrachloroguaiacol -
resorcinol -
phloroglucinol -
pyrogallol -
abietic acid -
eugenol -

catechol -
4-chlorocatechol -
4,5-dichlorocatechol -
3,5-dichlorocatechol -
3,6-dichlorocatechol -
3,4,5-trichlorocatechol -
tetrachlorocatechol -
3-methylcatechol -
4-methylcatechol -
m-hydroxyacetophenone -
p-hydroxyacetophenone -
m-methoxyacetophenone -
p-methoxyacetophenone -
acetovanillone -
acetoveratrone -
veratrole -
veratric acid -
o-vanillic acid -
p-vanillic acid -
benzoic acid -
p-hydroxybenzoic acid -
3,4-dihydroxybenzoic acid -

4-chloro-m-cresol -
trichloroethylene -
t-1,2-dichloroethylene -
dichloromethane -
chloroform -
carbon tetrachloride -
dibromomethane +
bromoform -
carbon tetrabromide -
glyoxal -
oxalic acid -
crotonic acid -
3-chlorocrotonic acid -
fumaric acid -
maleic acid -
chloromaleic acid -
muconic acid -
cis, cis-muconic acid -
mucochloric acid +
acetone -
chloroacetone -
1,3-dichloroacetone +
hexachloroacetone + ?

o-benzoquinone +?
tetrachloro-o-benzoquinone -
hydroquinone -
chlorohydroquinone -
2,5-dichlorohydroquinone -
p-quinone -
2,5-dichloro-p-quinone -
2,6-dichloro-p-quinone -
tetrachloro-p-quinone
trichloroacetic acid -
o-phenylenediamine
cis-3-chloroacrylic acid -
trans-3-chloroacrylic acid -

1,1,3,3-tetrachloroacetone +
tetrachloro-cis, cis-muconic acid -
benzaldehyde -
p-hydroxybenzaldehyde -
p-methoxybenzaldehyde -
2,3-dimethoxybenzaldehyde -
vanillin -
syringealdehyde -
3-chloro-4-hydroxybenzaldehyde -
3-chloro-2-hydroxybenzaldehyde -
3,5-dichloro-2-hydroxybenzaldehyde -
3,6-dichloro-2-hydroxybenzaldehyde +
hexachloro-1,3-butadiene -

Compounds tested at 10^3, 10^2, 10^1, 10^0 and 10^{-1} ug per plate.

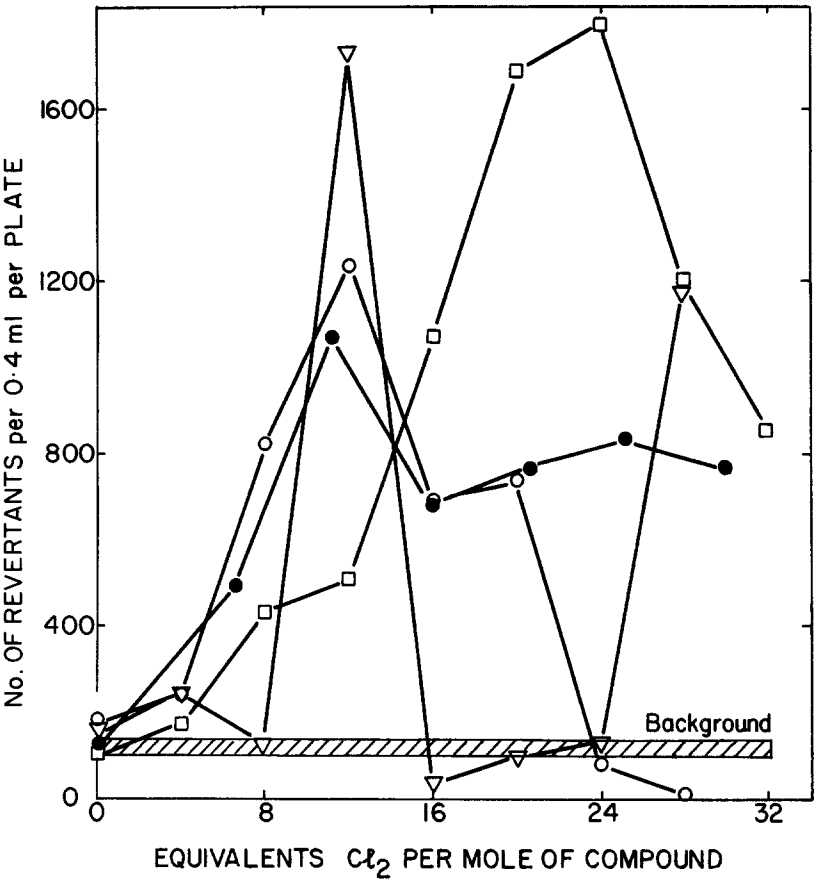

Figure 2. TA100 Ames revertants per plate versus equivalents of chlorine applied per mole of compound for four lignin model compounds

- □ acetoveratrone
- ▽ vanillin
- ● acetovanillone
- ○ syringealdehyde

So far, 43 pure compounds have been systematically chlorinated with a range of 0 to 32 equivalents of chlorine per mole of compound in 0.002 to 0.008 molar solution. In each case 0.4 ml of solution was applied per plate. All compounds shown in Table 2 produced mutagens on dilute aqueous chlorination except p-benzoquinone, tetrachlorocatechol, tetrachloroguaiacol, tetrachloro-ortho-quinone, abietic acid, acetone and caffeine, all of which produced toxicity, and glyoxal, benzoic acid and maleic acid, which produced neither mutagenicity nor toxicity.

TABLE 2

Mutagenicity on TA100 for compounds reacted with aqueous chlorine

	Peak Positions[1]	Revertants[2]	Toxicity[3]
lignin[4]	8	970	50
acetovanillone	11	880	NT
acetoveratrone	24	1685	80
m-hydroxyacetophenone	16	1400	90
p-hydroxyacetophenone	24	2240	90
m-methoxyacetophenone	12	1220	100
p-methoxyacetophenone	8	1240	80
phenol	12/32	750/1260	100/70
catechol	3/8	6000/ 980	NT/80
resorcinol	20	2000	100
hydroquinone	4	675	100
phloroglucinol	4/16/24	350/480/285	90/60/80
pyrogallol	2	180	100
3-methylcatechol	5	160	100
4-methylcatechol	8	220	100
benzaldehyde	2	140	100
p-hydroxybenzaldehyde	24	1930	100
p-methoxybenzaldehyde	12	1835	90
2,3-dimethoxybenzaldehyde	5	130	100
vanillin	12/28	1605/1035	100/90
syringealdehyde	12	1100	90
benzoic acid	none	none	100
p-hydroxybenzoic acid	17	380	40
3,4-dihydroxybenzoic acid	17	970	80
o-vanillic acid	17	1090	50
p-vanillic acid	12	1290	70
guaiacol	12/24	860/1200	100/80
veratrole	24	380	70
2,4,6-trichlorophenol	5	345	100
4,5-dichlorocatechol	30	2100	80
tetrachlorocatechol	none	none	50
tetrachloroguaiacol	none	none	40
p-benzoquinone	none	none	50
2,5-dichloro-p-benzoquinone	1	300	50
2,6-dichloro-p-benzoquinone	3	1460	90
tetrachloro-o-benzoquinone	none	none	80
abietic acid	none	none	NT
acetone	none	none	NT
eugenol	6	1400	NT
glyoxal	none	none	NT
fumaric acid	2	850	100
maleic acid	none	none	NT
muconic acid	2	900	NT

[1] Equivalents chlorine per mole of substrate at peak among peaks tested. [2] Revertants per 0.4 ml solution minus spontaneous revertants. [3] Percent bacterial survival at peak. [4] Alaska yellow cedar milled wood lignin supplied by W.G.Glasser, assumed molecular wt.180. NT-Not Tested.

It is rather interesting that chlorination of fumaric acid, the trans-isomer of butenedioic acid, produced a positive response to the Ames test on aqueous chlorination, while maleic acid, the cis-isomer did not. However, because of its very low solubility in water, fumaric acid was chlorinated in 50/50 volume/volume methanol/water. No mutagenicity was produced by chlorination in water or methanol alone.

Systematic variation in the structure of the substituted aliphatic groups and in the number of hydroxyl or methoxyl groups on the aromatic ring permits determination of the effect of such structural changes on mutagenicity and toxicity and this will be reported in subsequent papers.

The Ames test in this investigation is used as a diagnostic tool to try to identify the mutagens among the multitude of products and to establish the mechanism of their formation. Thus separated fractions can be tested to locate the mutagens. The number of equivalents of chlorine applied per mole of substrate to produce maximum mutagenicity permits maximizing the concentration of mutagens to aid in their separation and identification. The Ames test also permits study of alternative bleaching processes, such as substituting chlorine dioxide for chlorine which drastically decreases or eliminates the mutagenicity with all compounds tested. It also permits study of the methods of destroying the mutagens formed by chlorination. For example, raising the pH to the neutral range destroys some of the mutagens, but very high pH (10 -12) is required to destroy the more stable ones (Figure 3), showing that there are at least two types of mutagens formed by aqueous chlorination.

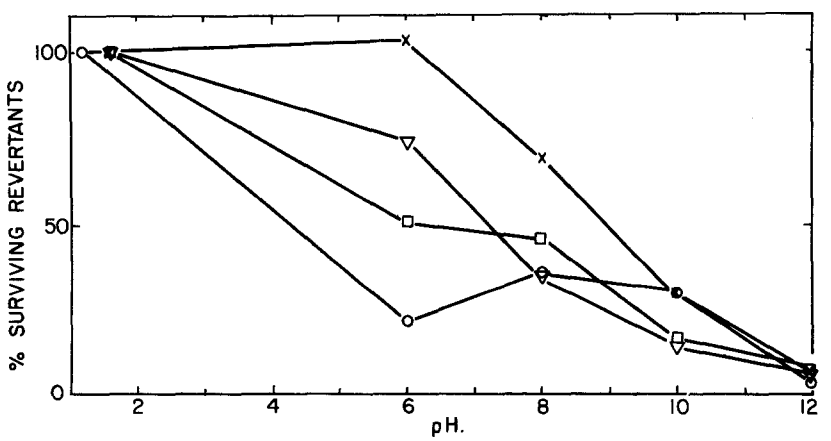

Figure 3. % mutagenicity remaining after raising the pH with NaOH to 6, 8, 10 and 12 for the times shown, and then reacidfying with HCl to pH 2.

× 7 equivalents of Cl_2 per mole of acetovanillone, 3 hrs at each pH

▽ 7.6% Cl_2 on unbleached kraft pulp; 1/2 hr at each pH

□ 7.6% Cl_2 on unbleached kraft pulp; 24 hr at each pH

○ 3equivalents of Cl_2 per mole of catechol; 3 hrs at each pH

When a nitrogen purge is used to remove residual chlorine, in some cases the solution remains highly toxic and therefore the Ames test is negative. In many cases titrating with sodium thiosulphate solution to destroy any chlorine residual destroys the toxicity and greatly enhances the mutagenicity.

Many plots of mutagenic activity versus equivalents of chlorine applied per mole of substrate show more than one peak, due either to destruction of mutagens or production of toxic substances in the valleys. It is possible that with the phenols the mutagens formed with the smaller ratio of equivalent chlorine are aromatic compounds, and with larger ratio are ring fracture aliphatic compounds such as substituted muconic acids or fumaric acids.

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