Another flame retardant, tris-(1,3-dichloro-2-propyl)-phosphate, and its expected metabolites are mutagens

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Another Flame Retardant, Tris-(1,3-Dichloro-2-Propyl)-Phosphate, and Its Expected Metabolites Are Mutagens

Abstract. *A flame retardant used in children's sleepwear, tris-(1,3-dichloro-2-propyl)phosphate (Fyrol FR2) is a mutagen in the* Salmonella-*mammalian tissue homogenate test after it has been activated by mouse or rat liver homogenate. The expected enzymatic hydrolysis product, 1,3-dichloro-2-propanol, is similarly a mutagen after activation by liver homogenate. A proposed metabolite of the flame retardant, 1,3-dichloro-2-propanone, is a potent mutagen in the absence of such activation. A flame retardant with similar structure, tris-(2,3-dibromopropyl)phosphate (tris-BP), was shown previously to be a mutagen, to cause sterility in animals, to be a carcinogen, and to be absorbed through human skin. These and other flame retardants have characteristic nuclear magnetic resonance spectra that can be used to determine which flame retardant is present in commercially purchased sleepwear. Sleepwear treated with tris-BP, Fyrol FR2, and other chemical additives was being sold in late 1977.*

In 1972 the United States established flammability standards for children's sleepwear. To comply with these standards, manufacturers began to use chemical additives (usually organic halogens or phosphate esters or both) to confer flame-resistant properties on the fabric. The most widely used of these flame retardants was the mutagen and carcinogen, tris-(2,3-dibromopropyl)phosphate (tris-BP) (*1*-*5*).

Although tris-BP is no longer being used to treat children's sleepwear, in late 1977 many millions of treated garments were still in storage (*6*) and some were still being sold. Other similar flame retardants are being used as replacements for tris-BP. We report here the finding that one of them which is structurally similar to tris-BP, namely tris-(1,3-dichloro-2-propyl)phosphate (Fyrol FR2) (*7*), and two of its expected metabolites, 1,3-dichloro-2-propanol and 1,3-dichloro-2-propanone, are mutagens (*8*).

In 1975, 6 to 10 million pounds (1 kilogram = 2.2 pounds) of Fyrol FR2 were produced in the United States (*9*). Although we have not been able to obtain production figures indicating what fraction of this amount was used to treat children's sleepwear, we have analyzed a selection of infants' pajamas in order to see whether Fyrol FR2 has replaced tris-BP as a flame retardant for polyester. Fyrol FR2 is used to treat garments made of polyester; tris-BP was used to treat both polyester and polyester blended with acetate or triacetate.

Children's pajamas (50 pairs) made of polyester and polyester blends (sizes 0 to 6X) were purchased in several states during the period from June through October 1977. A fabric sample from each garment was extracted in acetone and the resulting extracts analyzed by nuclear magnetic resonance (NMR). Typical NMR spectra of sleepwear extracts and flame retardants are shown in Fig. 1. Seventeen of the 50 pajamas, all of the polyester blend fabrics, were found to have been treated with tris-BP and nine of the polyester fabrics had been treated with Fyrol FR2, based on comparison of the NMR spectra of the extracts with those of the pure chemicals. Two of the other pajamas made of polyester had been treated with Mobil Antiblaze 19, a mixture of cyclic phosphate esters (*8*), and the remaining 22 pajamas showed no identifiable NMR spectrum. In addition to the NMR spectra attributable to the flame retardants, the sleepwear showed other peaks, mainly in the alkane region (< 3 parts per million), which are thought to be due to other chemical additives in the fabrics.

Prior to this work, Fyrol FR2 was negative in a mutagenicity test (*1*), in which polychlorinated biphenyl (PCB)-induced rat liver homogenates were used (*10*). However, a more thorough examination of Fyrol FR2 and its expected metabolites was undertaken because (i) Fyrol FR2 is very similar in structure to tris-BP; (ii) the structure of one expected metabolite, 1,3-dichloro-2-propanone, suggested it would be a potent alkylating agent and therefore a mutagen or carcinogen; and (iii) the structure of another expected metabolite, 1,3-dichloro-2-propanol, suggested it would be a mutagen after metabolic activation.

As illustrated in Fig. 2, Fyrol FR2 and dichloropropanol were weakly mutagenic on *Salmonella typhimurium* strain TA100 in the presence of phenobarbital-induced mouse liver homogenate. Dichloropropanone was strongly mutagenic on TA100 and required no activation. Each of six independent experiments with Fyrol FR2 with phenobarbital-induced mouse liver homogenate showed a dose response for mutagenicity. A repeatable dose response was also obtained in two experiments in the presence of PCB-induced mouse liver homogenate and in three experiments with phenobarbital-induced rat liver homogenate. The weakest response occurred with PCB-induced rat liver homogenate (*11*). Recently, a coded experiment on the mutagenicity of tris-BP and two samples of 96 percent Fyrol FR2 (supplied by Stauffer) confirmed these results. The mutagenicity of Fyrol FR2 was further confirmed in another laboratory in three tests in which PCB-induced mouse liver homogenate was used and in four tests in which PCB-induced hamster liver homogenate was used for activation (*12*, *13*).

Metabolic transformations of Fyrol FR2 to mutagens are proposed in Fig. 3 by analogy with the known metabolism of other related phosphotriesters (*14*). Oxidative *O*-dealkylation of Fyrol FR2 would be expected to produce 1,3-dichloro-2-propanone, which we show here to be a powerful direct-acting mutagen. A precedent for this would be the transformation of the pesticide chlorfenvinphos, which is oxidatively *O*-deethylated by liver microsomes in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen (*15*). Diisopropyl-1-naphthyl phosphate has been shown to undergo a similar dealkylation (*15*). Cleavage of the phosphate triester bonds in Fyrol FR2 by hydrolases in the cell would yield dichloropropanol, which we show here to be a mutagen in the presence of liver homogenate. Enzymes hydrolyzing phosphotriesters are found in many mammalian tissues (*14*). The glutathione *S*-transferases (*14*) would be expected to form glutathione adducts of Fyrol FR2, di-

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chloropropanone, or dichloropropanol, and these chlorine-containing glutathione conjugates could be alkylating agents and mutagens. With ethylene dichloride the glutathione adduct (a sulfur half-mustard) is a mutagen (*16*).

The different liver preparations used vary in their effectiveness in activating Fyrol FR2 to a mutagenic form. For the general screening of chemicals by means of the *Salmonella*-mammalian tissue homogenate test for mutagenicity, the use of about 20 to 50 μl of liver homogenate from rats injected with Aroclor 1254 (a PCB mixture) was recommended for activation (*10*). This standard procedure, which detects about 90 percent of a diverse sampling of carcinogens as mutagens (*17*), represents a compromise, however, and changing the variables pertaining to liver homogenate often results in higher levels of response: (i) a 20-μl amount of liver homogenate is too much for a maximum response with aflatoxin B1 and is too little for a maximum response with 2-acetylaminofluorene (*10*); (ii) PCB-induced rat liver homogenate is superior to phenobarbital-induced rat liver homogenate for benzo[a]pyrene and inferior for 2-acetylaminofluorene (*10*); (iii) rat liver homogenate gives a higher response than mouse liver homogenate for aflatoxin B1 (*18*) and a lower

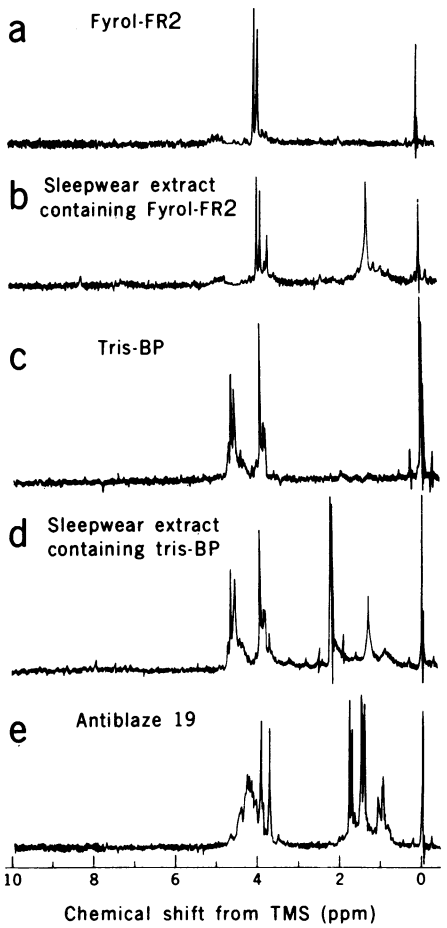

Fig. 1 (above left). Nuclear magnetic response spectra of (a) Fyrol FR2; (b) sleepwear extract containing Fyrol FR2; (c) tris-BP; and (d) extract of sleepwear containing tris-BP; (e) Antiblaze 19. A piece of each garment (100 cm^2) was extracted three times in 20 ml of acetone. If the fabric is a blend with acetate or triacetate it dissolves in the acetone. Therefore, to extracts of these fabrics we added an equal volume of hexane to precipitate the acetate. The precipitate was removed by filtration through Whatman No. 1 filter paper. The extracts were blown dry under nitrogen and the residues dissolved in 0.5 ml of deuterated chloroform with 1 percent tetramethylsilane (TMS) as a chemical shift marker. The NMR spectra were obtained on a Varian T60 spectrometer.

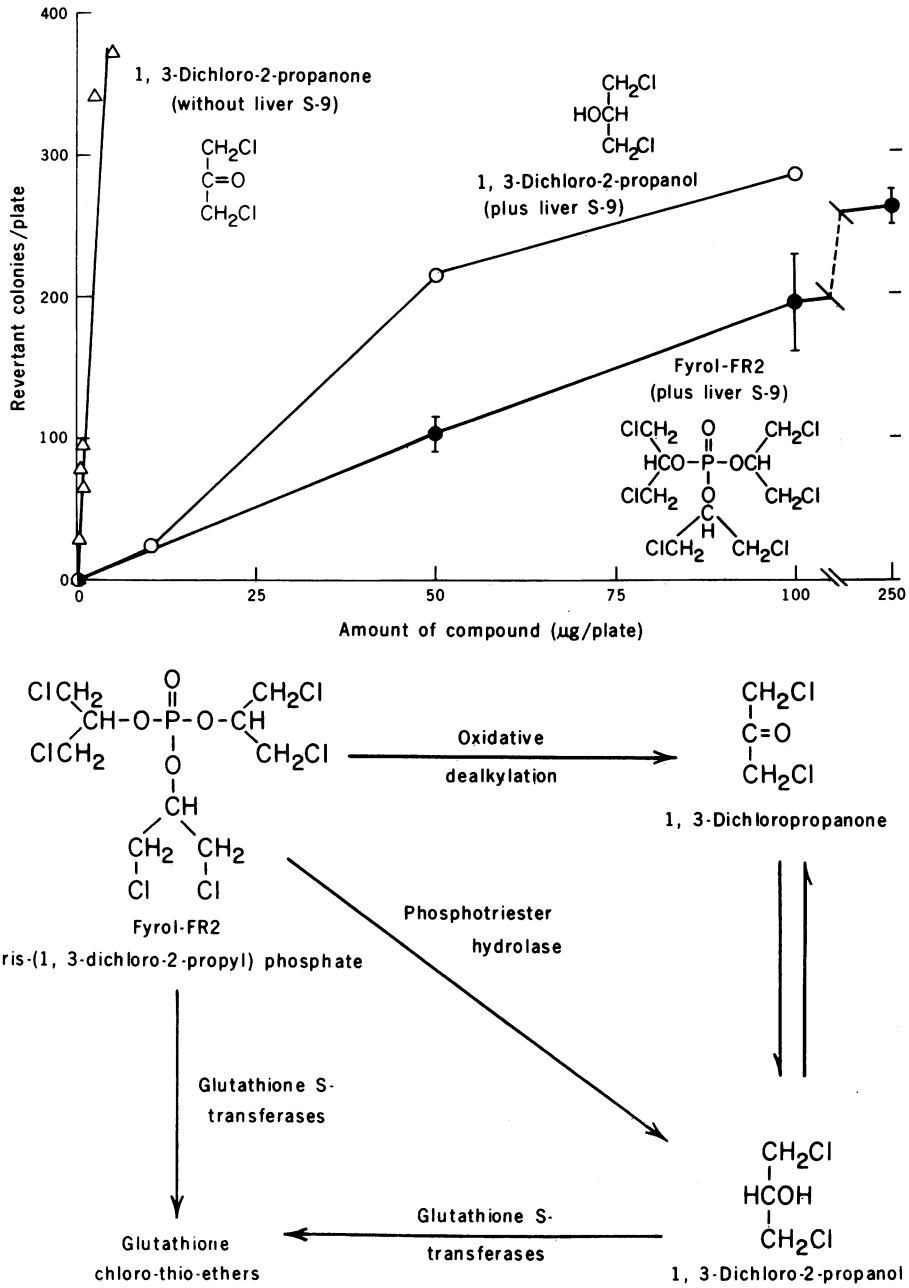

Fig. 2 (above right). Mutagenicity of Fyrol FR2, 1,3-dichloro-2-propanol, and 1,3-dichloro-2-propanone. All compounds were tested on *Salmonella typhimurium* strain TA100 as described (*10*). The number of spontaneous revertants (~150) has been subtracted from the revertant values plotted against the amount of mutagen. Where indicated, 20μl of phenobarbital-induced mouse liver homogenate (fraction S-9) was incorporated into the pour plates. Fyrol FR2 was tested in six independent experiments with 20 μl of phenobarbital-induced mouse liver homogenate (fraction S-9). All showed positive dose response curves, and the mean and standard errors are indicated. The controls for the six experiments had a mean of 156 ± 16 (standard error). The two different Fyrol FR2 samples (*8*) gave identical NMR spectra and mutagenicity results and have been averaged together. 1,3-Dichloro-2-propanol was less inhibitory to strain TA100 than Fyrol FR2; the mutagenicity kept increasing with dose (407 revertants per 250 μg; 937 revertants per 1000 μg). 1-3-Dichloro-2-propanol was also mutagenic in the presence of phenobarbital-induced rat liver homogenate, but not with PCB-induced rat liver homogenate. Fig. 3 (below right). Proposed metabolism of Fyrol FR2.

response for trichloroethylene (*12*). Thus for a more thorough test of a chemical, several liver preparations from different species and with various inducers should be tried. In the case of the Fyrol FR2, a weak mutagen in the test system, the mutagenicity was missed in the original study (*1*) because only the PCB-induced rat liver homogenate was used.

Tris-BP has been shown to be a mutagen in several test systems (*1*, *2*), to be a potent animal carcinogen (*3*), to be absorbed from fabric through human skin (*5*), and to cause testicular atrophy and sterility in animals (*4*). Fyrol FR2, a chlorinated chemical closely related to tris-BP in structure, is shown to be a mutagen in this report and thus is likely to be a carcinogen (*17*). Fyrol FR2 has also been found to be positive in a test for sister chromatid exchange, but its activity in this test is weak compared to that of tris-BP (*19*). Most nonpolar chemicals, including tris-BP and five other phosphotriesters, are absorbed through human skin (*20*); Fyrol FR2 is also likely to be absorbed. The first cancer test in experimental animals is in progress at the National Cancer Institute; the results will not be known for several years. Considering that alternatives to the use of dangerous flame-retardant additives are available (*2*), we recommend that these alternatives be used.

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8. Fyrol-FR2 (Stauffer, 99 percent pure) was obtained from D. J. Lisk of Cornell University, and commercial grade Fyrol-FR2 (96 percent pure) was supplied by Stauffer Chemical Company. The 1,3-dichloro-2-propanol was obtained from Aldrich Chemical Company; 1,3-dichloro-2-propanone from Eastman Company; and Antiblaze 19 (a mixture of cyclic phosphate esters) from Mobil.
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Spine Stems on Tectal Interneurons in Jewel Fish Are Shortened by Social Stimulation

Abstract. *Spined pyriform interneurons in community-reared jewel fish have more dendritic branches and spines in the deep tectal layers than those in isolates reared without visual-tactile contact with conspecifics. Furthermore, in the same dendritic loci in which the community-reared fish had more spines, the spine stems were shorter. The findings suggest that social stimulation induces localized formation of spines, which swell with synaptic activation. Shortening of the spine stem through elongated swelling of the spine head is likely to alter synaptic effectiveness through changes in electrotonic conductance.*

For years, the formation of new synaptic contacts or changes in conductivity of existing contacts has been hypothesized to be the basis of learning. In studies of mammals, social deprivation during rearing has been reported to reduce the receptive surface of neurons in relevant brain areas (*1*). Changes in synaptic density and terminal size, along with postsynaptic thickening, have also been reported (*2*). In a recent Golgi study, we found that cichlid fish deprived of seeing their own species have fewer dendritic spines and branches on specific tectal interneurons than do fish reared in community tanks (*3*). We also noticed that spines on the interneurons of socially deprived fish had long slender stems. In this report, the spine stem is defined as the thin stalk between the dendrite and the larger bulbous portion of the spine. Although spines have been reported to change shape somewhat with visual deprivation (*4*), to swell considerably during electrical stimulation (*5*), and possibly to have unusually long, thin stems in fetal and retarded humans (*6*), to our knowledge there are no reported data on the effects of restricted experience on stem length. We now report, from further study of the same material, that visual stimulation in the appropriate social context shortens spine stems, particularly in dendritic loci where spine density is also affected.

Jewel fish (*Hemichromis bimaculatus*, Gill 1862) obtained from the same spawn were reared in total isolation for 73 days and then assigned to isolate and control groups. Both groups experienced similar amounts of olfactory and auditory stimulation from nearby jewel fish and relatively equivalent amounts of background visual pattern stimulation from conspicuously colored graphics and other moving fish. Isolates and controls differed in that isolates could not associate these perceptual inputs with the configuration and behavioral repertoire of their own species. In place of jewel fish, the isolates were reared with blind cave characins (*Anoptichthys jordani*). They observed these eyeless unpigmented fish through the windows and permeable screens of their adjacent compartments, which were suspended in a flat 73-liter aquarium. The fish in the control group, on the other hand, were released in community tanks where they were in contact with conspecifics with which they frequently engaged in vicious territorial fighting.

Four adult females from each group, selected on the basis of size and weight, were killed in pairs at 402, 508, and 529 days (*7*). Whole brains were excised and

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