THE EFFECT OF BOTH POWDERED AND LIQUID RUBBER ADDITIVES ON THE GROWTH OF SOIL MICROORGANISMS

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Abstract: Two novel techniques were used to investigate rubber additive deterioration by soil microorganisms; over thirty additives were examined both for deterioration and toxicity.

Introduction

Rubbers are composed of a basic polymer, a vulcanizing system (usually zinc oxide, stearic acid, sulphur and an accelerator), and several other compounds including antioxidants, fillers, processing aids and pigments. Researchers who have reported the biodeterioration of compounded rubbers have generally not indicated which component of the rubber suffered attack, or the effects of other constituents on this attack. It has been shown that natural rubber polymers support mould growth (Williams 1982) however, it is difficult to estimate the biodeterioration of powdered additives as they cannot be easily recovered from soil. This paper describes the soil burial of rubber additives to determine those which are biodegradable, and those which exhibit toxic properties.

Materials and Methods

All chemicals used were commercially available and were used without further purification. The additives used in the investigation include accelerators (tetramethyl thiuram disulphide (TMTD), mercapto benzothiazole (MBT), cyclohexyl benzothiazole (CBS), Mercaptobenzothiazole, sulphonamide (MBTS) tetramethyl thiuram monosulphide (TMTM), zinc diethylcarbamate (ZDC), ethylene thiourea (Na 22) and diphenyl guanidine (DPG); antioxidants (2.2.4. trimethyl dihydroquinoline, octylated diphenylamine, 4-methyl-6-(dimethyl cyclohexyl) phenol, 2-2 methylene bis (6-1 methylcyclohexyl) paracresol); activators (zinc oxide, stearic acid, salicylic acid, flowers of sulphur); fillers (medium thermal carbon black, silica filler) and plasticisers and processing aids (dioctyl sebacate, dioctyl phthalate, mineral oil, paraffin jelly, paraffin wax, 75% pine tar, aromatic oil and golden factice.) Organisms were isolated and identified by their growth on malt extract or nutrient agar (Oxoid).

Soil burial experiments

Three techniques were used for exposing rubber additives to John Innes No 1 Soil, maintained as previously described (Williams 1982). The first technique involved placing powdered additives onto non-biodegradable polycarbonate membranes (Nucleopore Corporation), with a 12 μ pore diameter, and placing the membranes onto the soil with powders on the upper surface. The second technique involved embedding powdered additives onto a thin layer (0.3 cm) of epoxy resin (Araldite), poured onto aluminium foil. During the curing process an excess of powder was poured onto the resin. When cured, excess powder was shaken off the resin, which was then cut into 15 cm x 5 cm strips and placed with the additive impregnated face in contact with the soil. The third technique was an adoption of the technique of Rubidge (1974) in which water insoluble liquid additives were incorporated into agar medium using colloidal silica according to the method of Baruah *et al* (1967). Petri dishes containing the medium were then inoculated with 1 ml aqueous soil extract (3.0×10^7 organisms per ml) and sealed in cellophane to prevent drying, and incubated at 25°C.

Toxicity experiments

Additives which did not support microbial growth after three months soil burial were examined to determine if they possessed any biocidal or biostatic properties. Each additive was incorporated singly into nutrient agar, at varying concentrations, approximating the levels of incorporation in rubber formulations, and inoculated with soil extract (3.0×10^7 organisms per ml) and incubated at 25°C for 14 days. Toxicity levels were expressed as LD50 values.

Results

Soil exposure experiments (table 1)

After three months exposure to soil, both burial techniques used in the investigation yielded similar results for the twenty six compounds investigated. Using the soil burial and colloidal silica-agar techniques, it was found that accelerators, antioxidants and fillers were uncolonised by soil microorganisms but several plasticisers and processing aids did support mould growth, including stearic acid, salicylic acid, paraffin jelly, paraffin wax, pine tar and golden factice. The organisms isolated included *Penicillium pinophilum, P. variabile, Aspergillus ustus* and *A. niger*. Two species of *Bacillus* were found to grow on dioctyl sebecate and dioctyl phthalate.

Toxicity experiments (table 2)

Results show that all accelerators (with the exception of ZDC) were moderately or severely toxic to soil microorganisms when incorporated into nutrient agar at their working concentrations in rubber. Even ZDC exhibited some degree of toxicity at higher concentrations (1.5%). Two accelerators (DPG and MBT) completely retarded microbial growth of soil microbes at very low concentration (0.1%). Other additives investigated including antioxidants, activators and filler had no toxic effect on microorganisms with the exception of zinc oxide which gave slight bacteriostatic effects at a concentration of 5%.

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Table 1. *Growth and colonization of rubber compounds*

Compound	Class of Compound	Growth after 1 months	Growth after 3 months	Organism Isolated
TMTD (tetramethyl thiuram disulphide)	Accelerator	0	0	–
MBT (mercaptobenzothiazole)	"	0	0	–
CBS (cyclohexyl benzothiazole)	"	0	0	–
MBTS (mercaptobenzothiazole sulphenamide)	"	0	0	–
TMTMS (tetramethyl thiuram monosulphide)	"	0	0	–
ZDC (zinc salt tetraethyl thiuram disulphide)	"	0	0	–
DPG (diphenyl guanidine)	"	0	0	–
Na22 (ethylene thiorea)	"	0	0	–
'Flectol' H (polymerised 2,2,4-trimethyl dihydro quinolins	Antioxidants	0	0	–
'Octamine' (octylated diphenylamine)	"	0	0	–
'Permanax WS' 2,2-methylene bis 6-1 methyl cyclohexyl) paracresol	"	0	0	–
'Permanax WSL' 4,methyl-6-(dimethylcyclohexyl) phenol	"	0	0	Slight growth of *Flavobacterium* sp
'Permanax BL'	"	0	0	–
Zinc Oxide	Activators	0	0	–
Stearic Acid	"	++	++	Variety of microorganisms
PVI Santogard	Pre-vulcanisation Inhibitors (retarders)	0	0	–
Salicylic Acid	"	+++	+++	*Penicillium pinophilum*
Sulphur-Magnesium Coated		0	0	–
Flowers of Sulphur		0	0	–
DOS (dioctyl sebacate)	Plasticisers and processing aids	0	++	Slight hyphal growth. Bacteria isolated
OM 13 (mineral oil)	"	0	0	–
DOP (dioctyl phthalate)	"	0	+	No fungal growth, Bacteria isolated
Paraffin Jelly - soft yellow	"	+++	+++	*Penicillium pinophilum* *Penicillium* variable
Paraffin Wax	"	+++	+++	*Penicillium pinophilum* (heavy growth)
75% Pine Tar (on inert filler)	"	+	+++	*Aspergillas ustus* (sporulating growth)
"Dutrex 729" (Aromatic Oil)	"	0	0	–
Golden Factice	"	+	++	*Penicillium pinophilum*
Medium Thermal Carbon Black	Fillers	0	+	V. slight growth (hyphal)
High Abrasion Carbon Black	"	0	+	V. slight growth (hyphal)
Hard Grade A Clay	"	0	0	–
Precipitating Whiting Chalk	"	0	0	–
Silica Filler (VN3)	"	0	0	–

0 = no growth + = slight growth <25% ++ = 25%-50% coverage +++ = above 50% coverage

Table 2. *Toxicity of various additives to soil microorganisms*

Compound	Class	LD50 4 Day	LD50 14 Day	Degree Toxicity
Tetramethyl thiuram disulphide (TMTD)	accelerator	< 0.1%	< 0.1%	highly toxic
Tetramethyl thiuram monosulphide (TMTM)	accelerator	< 0.1	< 0.1%	highly toxic
Mercaptobenzothiazole (MBT)	accelerator	⩽ 0.1%	⩽ 0.1%	highly toxic
Mercaptobenzothiazole sulphonamide (MBTS)	accelerator	0.5%	0.75%	moderately toxic
Diphenyl guanidine (DPG)	accelerator	⩽ 0.1%	⩽ 0.1%	highly toxic
Cyclohexyl benzothiazole (CBS)	accelerator	0.25%	0.25%	moderately toxic
Ethylene thiourea (Na22)	accelerator	1.0%	1.0%	moderately toxic
Zinc diethylcarbamate (ZDC)	accelerator	0.75%	> 1.5%	poor toxicity
2,2,4 trimethyl dihydroquinoline	antioidant	> 1.5%	> 1.5%	non-toxic
Octylated diphenylamine	antioxidant	> 1.5%	> 1.5%	non-toxic
4-methyl-6 (dimethylcyclohexyl) phenol	antioxidant	> 1.5%	> 1.5%	non-toxic
Zinc oxide	activator	5.0%	> 5.0%	slightly toxic
Flowers of sulphur	activator	> 5.0%	> 5.0%	non-toxic
Carbon black	filler	> 30%	> 30%	non-toxic
Silica filler	filler	> 20%	> 20%	non-toxic

Discussion

The results presented in this paper, utilizing two novel techniques of soil burial, confirm previous observations that accelerators are not colonised by soil microorganisms and are biocidal at concentrations used in rubber formulations; antioxidants and fillers are also not colonized, but are non-toxic to soil microorganisms, and that plasticisers and processing aids are often capable of supporting microbial growth. Soil burial techniques have previously been applied exclusively to solid materials, especially in the form of sheets and films, and although it is possible to incorporate powders into soils it is subsequently not possible to examine visually the growth of microbes *in situ*. The techniques employed here, particularly the use of an inert resin support, enables such an examination to be carried out. However, this type of experiment is inadequate to predict the effect of various compounding ingredients on the microbial deterioration of rubber, as they do not take into account those chemical changes which occur during vulcanization. Future work will include investigation into the biodeterioration of rubber vulcanizates, and the effect of additives upon this deterioration.

References

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Received September 1983