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In Vivo and *in Vitro* Biological Effects of the Flame Retardants Tris(2,3-dibromopropyl) phosphate and Tris(2-chlorethyl)orthophosphate*

M. SALA,† Z. G. GU,‡ G. MOENS and I. CHOUROULINKOV
Institut de Recherches Scientifiques sur le Cancer CNRS, B. P. No. 8-948002 *Villejuif Cedex, France*

Abstract—*Tris(2,3-dibromopropyl)phosphate (Tris-BP) and Tris(2-chloroethyl) orthophosphate (Genomoll P) were analyzed for mutagenic and carcinogenic activity in several* in vitro *and* in vivo *mammalian systems. In the* in vitro *tests both Tris-BP and Genomoll P increased sister chromatid exchanges in V79 cells with a dose–response relationship for Tris-BP. The mutation assays using the same cells (HGPRT locus) were negative. Both compounds showed positive results in the transformation of Syrian hamster embryo cells. A very low frequency of transformation in the C3H10T1/2 cells was obtained; we consider this result essentially negative. In the* in vivo *assays Tris-BP gave positive and Genomoll P questionable results in the micronucleus test performed on Chinese hamsters. Both gave negative results in short-term skin tests. In the long-term skin tests, Tris-BP showed an initiating activity which was not observed with Genomoll P. When they were used as promoters both chemicals increased the incidence of lung adenomas in mice. Comparatively, Genomoll P is far less hazardous than Tris-BP.*

INTRODUCTION

THE MOST widely used flame-retardant additive for children's nightwear has been Tris(2,3-dibromopropyl)phosphate (Tris-BP). Studies on Tris-BP have shown that it is mutagenic in bacteria [1–5], in *Drosophila* [6] and in L5178Y mouse lymphoma cells [5]. It is carcinogenic when fed to mice and rats [7, 8] and has induced benign and malignant tumours of the skin, forestomach and oral cavity in mice following skin application [9]. Tris-BP has also been shown to induce unscheduled DNA synthesis in human cells [10] and to transform mouse cells in culture [5].

Tris-BP has been reported to induce a significant dose-dependent increase in sister chromatid exchanges (SCEs) in V79 cells, either in culture or in diffusion chambers implanted into mice [11], and in L5173Y mouse lymphoma cells [5].

This substance has not therefore been marketed since 1977. Tris(2-chlorethyl)orthophosphate (Genomoll P), which is used as a flame retardant in expanded polyurethane thermal insulation of buildings, could be used also as a replacement of Tris-BP. It was tested in bacteria [2, 4], but was not mutagenic. Very few studies have been published, however, on the mutagenic and carcinogenic potential of Genomoll P in mammalian systems.

The present report describes the comparative results obtained in mutagenicity, transformation and mouse skin carcinogenicity tests with Tris-BP and Genomoll P.

MATERIALS AND METHODS

Materials

The following chemicals were obtained from the sources listed: Tris(2,3 dibromopropyl)phosphate (Tris-BP), density = 2.2, Ugine Kuhlmann; Tris(2-chlorethyl)orthophosphate (Genomoll P), density = 1.42, Hoechst; benzo(a)pyrene (BaP), Fluka; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), Sigma; 3-methylcholanthrene (MCA),

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†To whom requests for reprints should be addressed.
‡Present address: School of Public Health, Sanghai First Medical College, Shanghai 200032, People's Republic of China.

Fluka; methyl methanesulfonate (MMS), Janssen Pharmaceutica; 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), P. Borchert, 7752 Mitchell Road, Eden Prairie, MN 55344, U.S.A.; thioguanine (TG), Aldrich; D-glucose-6-phosphate, Sigma; β-nicotinamide adenine dinucleotide phosphate, Sigma; colchicine (Colchineos), Houdé; H 33258, Riedel De Haën.

Animals

Male and female Chinese hamsters (40 g mean weight) from the local breeding colony were used for the micronucleus test. Female Swiss mice (SPE local breeding house) were used for the skin tests: 45 ± 2 days old or 60 days old for short-term and long-term skin tests respectively.

In vitro *studies*

Induction of forward gene mutation in V79 cells. The induction of 6-thioguanine-resistant (TG_r) mutants was carried out according to the method of Abbondandolo *et al.* [12] using V79 Chinese hamster lung fibroblasts that were obtained from IARC, Lyon.

A 9000-*g* supernatant was prepared from the livers of 280–300 g male Wistar rats that had been injected (i.p.) with 40 mg/kg of MCA in olive oil 48 hr before death. The livers were washed with phosphate-buffered saline (PBS, pH 7.4) containing 1.15% KCl and homogenized in the same buffer (1 g wet wt liver in 5 ml PBS). The homogenate was centrifuged at 9000 *g* for 20 min at 4°C. The supernatant (S9) was used immediately after addition of cofactors (for 5 ml of S9, 3 mg $MgCl_2$, 20 mg glucose-6-phosphate and 3 mg NADP). The proportion of S9 in the test system was expressed as a percentage of the volume: therefore 20% of S9 represents a mixture of 1 ml S9 and 4 ml Dulbecco's modified Eagle's MEM (D-MEM).

For the experiments, 2×10^6 cells were plated in 25-cm^2 tissue culture flasks containing 5 ml D-MEM supplemented with 6% heat-inactivated foetal bovine serum (FBS, GIBCO) and incubated in a humidified incubator at 37°C in an atmosphere of 10% CO_2 in air. Twenty-four hours later the medium was removed, the cells were washed with PBS and reincubated in 5 ml D-MEM (without serum) containing the substance to be tested, which had been added as a solution in acetone (final concentration 0.5%) in the presence or absence of S9. After different times of treatment the cells were trypsinized and replated in 4 100-mm diameter dishes at 5×10^5 cells/dish for mutation studies and in 6 60-mm diameter dishes at 100 cells/dish for measurement of survival. For the following expression time 2.5×10^5 cells were plated in 3 or 6 dishes.

An identical procedure was repeated on different days following treatment to give different expression times. TG 5 μg/ml was added 3 hr after plating at each expression time. The dishes were stained with Giemsa after 8 days to measure survival and after 10 days to estimate mutations. Acetone, MNNG and BP were tested simultaneously as negative and positive controls.

Sister chromatid exchanges (SCEs) in V79 cells. For the experiments, 1.0×10^6 cells were plated into 100-mm dishes containing 10 ml Eagle's MEM (GIBCO) supplemented with 10% heat-inactivated FBS and incubated for 16 hr in a humidified incubator at 37°C in an atmosphere of 5% CO_2 in air. The medium was then replaced with FBS-free MEM containing the test substance, that had been added as a solution in acetone, at various doses. After treatment for either 1 hr without S9 or for 2 hr with S9 (20%), the medium was replaced with complete medium containing 5-bromodeoxyuridine (BrdU, 5 μg/ml) for 7 hr in order to cover one S-phase. The BrdU-containing medium was then replaced by complete medium and the cells incubated for another 18 hr. All the dishes were incubated in darkness. Colchicine (30 μg/ml) was added to the media 2 hr before the cells were collected in order to accumulate metaphases. Cells were then trypsinized and treated as described by Pera and Mattias [13] to allow recognition of SCE between differentially stained chromatids. The mean number of SCEs was determined on the basis of 30 intact metaphases unless otherwise indicated.

Transformation of C3H10T1/2 cells. C3H10T1/2 cells were obtained from Dr. C. Heidelberger's laboratory (University of Southern California Comprehensive Cancer Center, Los Angeles, CA) and transformation assays were carried out according to the method of Mondal *et al.* [14].

Two thousand cells were plated for transformation assays and 100 cells were plated for the determination of plating efficiency in 60-mm dishes containing 5 ml Eagle's Basal Medium (BME, GIBCO) supplemented with 10% heat-inactivated FBS and incubated in a humidified incubator at 37°C in 5% CO_2 in air. As shown in the experimental schedule in Table 4, the medium was replaced 24 hr after plating with complete medium containing MCA or the substances to be tested that had been added as a solution in acetone (0.5% final concentration in the medium) and either with or without S9. The dishes containing S9 (13%) were incubated for 3 hr and the dishes without S9 for 24 hr, and then the medium was replaced with fresh complete medium. Twice weekly before the cells became confluent, then once weekly thereafter, the medium was replaced with fresh medium.

The dishes were stained after 8 days (plating efficiency) or after 40 days (transformation). The numbers of type 3 foci were scored.

Transformation of Syrian hamster embryo cells. The transformation assay was carried out according to the method of Di Paolo [15]. Primary cultures were prepared from decapitated and eviscerated embryos and grown in Dulbecco's modified Eagle's Minimum Essential Medium (containing 4.5 g/l glucose) supplemented with 10% foetal bovine serum (FBS). Three hundred cells from 2-day-old secondary cultures of hamster embryo cells were plated into 60-mm plastic Petri dishes containing 4 ml of complete medium supplemented with 15% serum and 6×10^4 X-irradiated Syrian hamster feeder cells. The cells were treated 24 hr after seeding: test substances, prepared as solutions in acetone, were added in 2 ml of complete medium so that each dish contained a total of 6 ml of medium. Control cultures were similarly treated with complete medium containing a final concentration of 0.5% acetone. The dishes were kept in incubators at 37°C in an atmosphere of 10% CO_2 in air and the medium was not changed during the course of the experiment. Seven days later the colonies were fixed, stained and examined for the presence of morphologically transformed colonies. Transformation was characterized by the random orientation of cells in colonies that contained piled up cells that were not seen in the controls.

In vivo *studies*

Micronucleus test. Experiments were carried out in Chinese hamsters according to the method of Schmid [16], with slight modifications.

The chemicals tested were dissolved in dimethylsulfoxide (DMSO) and foetal bovine serum was added to the DMSO solution in order to obtain the required concentrations; the final concentration of DMSO in the mixture was less than 20%.

Test substances were given at different doses as a single intraperitoneal injection. Bone marrow samples were obtained 24 hr later by flushing the marrow canal of the femur into a centrifuge tube. The cells were suspended by gentle pipetting and centrifuged at 150 *g* for 5 min. The sediment was spread on to slides that were dried at room temperature for 24 hr and then stained using May-Gruenwald and Giemsa.

Two thousand polychromatic erythrocytes per animal were analysed for the presence of micronuclei. To compare the mean values of micronuclei obtained Student's *t*-test was used, and in order to obtain estimates of the dose-response linear regression analysis was applied.

Short-term tests on mouse skin. The *in vivo* short-term skin tests for sebaceous gland suppression and for the induction of epidermal hyperplasia were carried out according to the methods previously published [15]. Groups of Swiss mice at 45 days of age received dorsal applications on days 1, 3 and 5 of Tris-BP or of Genomoll P, which were applied as solutions in acetone (0.05 ml) at 3 different dose levels (Table 6). The control groups were treated with BP, TPA or acetone respectively. The treated skin areas were removed on day 8 and the number of sebaceous glands and the thickness of the epidermis were measured by standard procedures [17].

Long-term skin tests. Female 60-day-old Swiss mice were randomized into groups of 35. The animals were then housed in individual cages for the duration of the experiments in order to prevent inter-mouse licking.

For initiation a single application of DMBA (50 μg), Tris-BP (110 mg) or Genomoll P (71 mg) was made as a solution in acetone (0.1 ml) to the dorsal skin of mice that had been closely clipped 48 hr earlier.

It was not necessary to include solvent controls because the background of skin and other tumors is defined by our historical data based on more than 10,000 mice.

In the tumour promotion studies the mice received, for 78 weeks, twice weekly applications, to the same area of dorsal skin, of an acetone solution (0.05 ml) of TPA (1 μg), Tris-BP (33 mg) or Genomoll P (21 mg), started 1 week after initiation.

In order to test these three substances for their ability to act as complete carcinogens, a second series of mice received the same twice weekly applications as the promoted mice but without any initiation treatment.

The total doses applied to the dorsal skin of the mice were TPA, 156 μg/mouse, Genomoll P, 3.3 g/mouse, and Tris-BP, 5.1 g/mouse. All applications to the skin were made with the aid of an accurate, automatic microvolumetric dispenser. The animals were examined regularly and the time of appearance of papillomas and of malignant neoplasms on the skin were recorded. Systematic post-mortem and histological examinations were performed on all animals.

RESULTS

Mutagenicity in V79 cells

Table 1 gives the frequency of TG_r mutants obtained in the controls and in the groups treated with Genomoll P and Tris-BP without a liver microsome-activation system. The results show that Genomoll P and Tris-BP do not significantly

increase 6-thioguanine-resistant mutants. This was confirmed in several independent experiments. Using a rat activation system (S9 fraction), we also obtained negative results not reported here.

SCE induction

The results presented in Table 2 show that the test substances significantly increase SCEs. A dose-response relationship was observed for Tris-BP. Genomoll P induced SCEs but no clear dose response was obtained. Genomoll P was toxic at 3000 μg/ml, the limit of solubility, and mitosis was partially inhibited at this concentration.

Transformation in C3H10T1/2 cells

The ability of each test compound to induce transformation is shown in Table 3. Transformed type 3 foci were obtained in C3H10T1/2 cells with MCA treatment (1 μg/ml). When the cells were treated with Tris-BP, with or without S9 fraction, and were then treated several times with TPA (0.1 μg/ml), a very low rate of transformation was observed (at the concentration of 80 μg/ml without S9, 1 type III focus out of 20, and at the concentration of 40 μg/ml with S9, 1 type III focus out of 33). Genomoll P in the presence of S9 also gave a very low incidence of transformed foci (1 type III focus out of 42).

Transformation of Syrian hamster embryo cells

The results of tests for the transformation of Syrian hamster embryo cells are presented in Table 4. Positive results were obtained with both Tris-BP and Genomoll P. Tris-BP at concentrations between 25 and 40 μg/ml gave a high level

Table 1. Induction of 6-thioguanine-resistant mutants by Tris-BP and Genomoll P in V79 cells

Compound	Concentration μg/ml	Mutants per 10^6 survivors at expression time: 0 days	3 days	6 days
Tris-BP	37.5	15.2	11.4	22.4
Tris-BP	75.0	11.1	10.9	18.4
Tris-BP	150.0	10.0	10.8	17.6
Genomoll P	500.0	4.8	14.6	7.4
Genomoll P	1000.0	16.1	10.8	6.4
Genomoll P	2000.0	4.7	8.4	3.5
Acetone	0.5%	7.9	5.5	12.9
MNNG	0.5	17.1**	402***	399***

*P <0.05; **P <0.01; ***P <0.001.

Table 2. Induction of sister chromatid exchanges (SCEs) in V79 cells after exposure to Tris-BP and Genomoll P (mean values from 30 metaphases scored ± standard error)

	Compound	Concentration, μg/ml	S9-mix, 0%	S9-mix, 20%
	Acetone	0.5%	5.57 ± 0.41	7.13 ± 0.43
	BaP	1.0	—	9.13 ± 0.59***
	Tris-BP	17.2	7.53 ± 0.67**	
	Tris-BP	24.5	7.33 ± 0.53	7.13 ± 0.64
Exp.I	Tris-BP	35.0	9.47 ± 0.76***	—
	Tris-BP	50.0	12.00 ± 0.99***	9.10 ± 0.54**
	Genomoll P	343.0	6.80 ± 0.54	—
	Genomoll P	490.0	7.00 ± 0.65	8.47 ± 0.67
	Genomoll P	700.0	8.23 ± 0.63***	9.73 ± 0.64***
	Genomoll P	1000.0	7.40 ± 0.64*	
	Acetone	0.5%	4.57 ± 0.31	—
Exp. II	Tris-BP	100.0†	11.79 ± 0.77	—
	Tris-BP	200.0	toxic	—
	Genomoll P	2000.0	7.00 ± 0.61**	—
	Genomoll P	3000.0‡	9.00 ± 1.43***	—

*P <0.05; **P <0.01; ***P <0.001.
†Only 14 metaphases scored (toxic).
‡Only 9 metaphases scored (toxic).

Table 3. Transformation of C3H10T1/2 cells induced by Tris-BP, Genomoll P and MCA

Compound	Treatment Concentration, μg/ml	S9†	Treatment* in each medium change, μg/ml‡	Plating efficiency, %	No. of dishes with type III foci/total No. of dishes§	Percentage of dishes with type III foci
Acetone	0.5%	—	—	27	0/51	0
MCA	1.0	—	—	30	19/41	46.3
Tris-BP	40.0	—	—	19.6	0/34	0
Tris-BP	40.0	+	—	20	0/25	0
Tris-BP	40.0	—	TPA (0.1)	21	0/18	0
Tris-BP	40.0	+	TPA (0.1)	17.6	1/33	3.0
Tris-BP	80.0	—	—	7.3	0/18	0
Tris-BP	80.0	—	TPA (0.1)	10	1/20	5.0
Genomoll P	900.0	—	—	17	0/39	0
Genomoll P	900.0	+	—	16	1/42	2.3
Genomoll P	900.0	—	TPA (0.1)	23	0/19	0
Genomoll P	900.0	+	TPA (0.1)	16.6	0/28	0
Genomoll P	1500.0	—	—	10.3	0/19	0
Genomoll P	1500.0	—	TPA (0.1)	11.7	0/17	0
—	—	—	TPA (0.1)	31.7	0/16	0

*Twenty-four hours after plating.
†13% of S9-mix.
‡Three days after the first 24-hr treatment.
§The number of dishes containing type 3 foci were scored 40 days after plating.

Table 4. Transformation of Syrian hamster embryo cells by Tris-BP, Genomoll P and BaP

Chemical	Concentration, μg/ml	No. of dishes	Cloning efficiency, %	No. of transformed colonies/dish	Percentage of colonies transformed
Acetone	0.5%	10	22	0	0
BaP	1	10	14.3	1	2.3
Tris-BP	25	10	17	2.1	4.1
	30	10	17.4	1.8	3.4
	35	10	15.2	1.6	3.5
	40	10	12.6	2.1	5.5
Genomoll P	400	10	14	1.4	3.3
	500	10	15.7	0.6	1.2
	600	10	11.5	0	0
	800	10	4.7	0	0

of transformation. Genomoll P also gave a high level of transformation but the concentrations used were 10–20 times higher than those with Tris-BP. If the results are expressed as transformed colonies per μg of tested compounds, the transforming activities are in the order: BaP, Tris-BP and Genomoll P, which give respectively 0.166, 0.014 and 0.00058 colonies per μg.

Micronucleus induction

The results (Table 5) show that Tris-BP significantly increases the micronuclei at the dose of 400 mg/kg with a dose–response relationship for 400 and 800 mg/kg. A slight effect is found with Genomoll P, but due to the different response between sexes and variations with the doses the interpretation of such results is difficult.

Short-term skin tests

The result presented in Table 6 show that in mouse skin treated with Genomoll P or Tris-BP sebaceous glands were not suppressed and that hyperplasia was not induced.

Long-term skin tests

The results presented in Table 7 show that Tris-BP does not have any effect as a complete carcinogen on uninitiated mouse skin (group 1) at the concentration used, which resulted in a total dose of 5.1 g/animal, which is substantial. The same dose, when applied to mice initiated with DMBA, showed no tumour-promoting effect (group 3) on the skin. However, the incidence of lung adenomas was significantly increased. Since this occurred in both groups 1 and 3 it indicates that DMBA as the initiator did not play a role in the induction of these type of tumours. The incidence of skin tumours in mice initiated with Tris-BP (group 2) is significantly increased compared to the control (group 7). The incidence of lung adenomas (group 2) is low due to the small single dose of Tris-BP used (compared to the

Table 5. Micronuclei (MN) induced in polychromatic erythrocytes (PE) from Chinese hamsters treated with Tris-BP and Genomoll P

Compound	Dose, mg/kg	No. of animals	MN (n/1000 PE ± S.E.) per: sex	groups
Tris-BP	200.0	2♂	5.50 ± 0.65	4.75 ± 0.56
		2♀	4.00 ± 1.63	
Tris-BP	400.0	2♂	10.50 ± 1.85*	8.38 ± 1.24**
		2♀	6.25 ± 0.86**	
Tris-BP	800.0	2♂	10.50 ± 1.19**	10.75 ± 0.96***
		2♀	11.00 ± 1.69**	
Genomoll P	62.5	2♂	4.00 ± 0.58	5.25 ± 0.70
		2♀	6.50 ± 0.96*	
Genomoll P	125.0	2♂	6.25 ± 0.75	6.63 ± 0.57***
		2♀	7.00 ± 0.92**	
Genomoll P	250.0	2♂	7.25 ± 0.25*	7.00 ± 1.00**
		2♀	6.75 ± 2.14	
DMSO	2200.0	2♂	4.00 ± 0.92	3.50 ± 0.50
		2♀	3.00 ± 0.41	
MMS	20.0	2♂	82.75 ± 2.43***	70.63 ± 5.30***
		2♀	58.50 ± 9.24***	

*P <0.05; **P <0.01; ***P <0.001.

Table 6. Suppression of sebaceous glands and induction of epidermal hyperplasia in mouse skin treated with Tris-BP and Genomoll P

Treatment	Dose†, mg	No. of mice	Sebaceous glands‡	Epidermal hyperplasia‡
Tris-BP	49.5	25	9.95 ± 2.91	16.53 ± 2.98
	82.5	25	11.30 ± 2.20	17.40 ± 3.00
	115.5	25	10.80 ± 3.85	16.70 ± 2.94
Acetone	0.15 ml	25	10.10 ± 3.71	15.25 ± 2.63
BaP	0.075	25	4.79 ± 3.00*	17.90 ± 3.40
TPA	0.003	25	4.20 ± 1.91**	65.2 ± 3.52***
Genomoll P	31.9	25	16.0 ± 2.32	12.8 ± 0.95
	53.2	25	15.5 ± 2.08	12.7 ± 0.46
	74.5	25	16.8 ± 1.63	12.2 ± 0.36
Acetone	0.15 ml	24	13.7 ± 1.87	11.8 ± 0.50
BaP	0.075	25	9.32 ± 1.59***	12.5 ± 0.40*

*P <0.05; **P <0.01; ***P <0.001 when the means were compared with the negative control means.
†Total dose applied in 3 applications, each of which contained the test compound as a solution in 0.05 ml acetone.
‡Data from 12 microscopic fields.

multiple dose applied in groups 1 and 3). We can say, therefore, that the incidence of lung adenomas seen in groups 1 and 3 is probably dose-dependent.

Genomoll P shows no complete carcinogenic or promoting activity on mouse skin (groups 4 and 6). It might have a weak initiating effect, but the data are not statistically significant. Genomoll P also increases the incidence of lung adenomas in group 4 but not in group 6. It would seem that DMBA may reduce the effect of Genomoll P in inducing lung adenomas. The same tendency was found using Tris-BP (groups 1 and 3).

The incidence of other tumours varies between the groups, but these differences are included in the variability of individual mice and they cannot be attributed to the different treatments.

DISCUSSION

Tris-BP (17.2–100 μg/ml) and Genomoll P (700–1000 μg/ml) showed activity in the SCE test

Table 7. Development of tumours in female Swiss mice in initiation–promotion experiments carried out with Tris-BP and Genomoll P

Group	Treatment: Initiation	Treatment: Promotion or repeated treatment	No. of mice	No. of mice with tumours: Skin tumours	Lung adenomas	Other tumours: No.	Other tumours: Type
1	—	Tris-BP†	33	0	14	3	two lymphosarcoma, hepatoma
2	Tris-BP	TPA‡	34	26§	7	0	
3	DMBA	Tris.BP†	33	3	11	2	mammary tumour, perianal carcinoma
4	—	Genomoll P‖	32	0	12	2	hepatoma endometrial sarcoma
5	Genomoll P	TPA‡	33	17§	7	1	hepatoma,
6	DMBA	Genomoll P‖	32	0	6	6	lymphosarcoma, mammary tumour, hepatoma, uterine angioma, endometrial sarcoma, perivular papilloma.
7	—	TPA‡	28	12	5	0	

†Tris-BP: total dose, 5.1 g/animal (170 g/kg bw).
‡TPA: total dose, 156 μg/animal.
§Two squamous cell carcinomas in each group.
‖Genomoll P: total dose, 3.2 g/animal (109 g/kg bw).

either in the presence or in the absence of the S9 activation system: we have found that the doses which double the SCE frequency of the control are 50 μg/ml and 3000 μg/ml for Tris-BP and Genomoll P respectively.

Furukawa *et al.* [11] also obtained positive results for Tris-BP using the same system in the absence of S9, and Brusick *et al.* [5] found a significant dose-related increase in SCE in mouse cells when rat liver S9 was added.

Tris-BP induced micronuclei in mouse bone marrow. As far as the statistical analysis was possible Genomoll P gave positive results. Further data in the same or in another species are needed.

The results of the transformation assay using C3H10T1/2 cells show that Tris-BP and Genomoll P produced a very low frequency of transformation and that they were essentially negative in this respect.

The negative results obtained in the mutation assay with V79 cells could be explained by the fact that our MCA-induced rat activation system is not appropriate since the positive control (BaP) showed a great variation between experiments. Brusick *et al.* [5] found a positive result with Tris-BP in the gene mutation assay using L5178Y mouse lymphoma cells with the S9 fraction of Aroclor-induced rat liver.

From the *in vivo* results, Tris-BP might have been expected to show initiating activity *in vitro*. However, in the C3H10T1/2 system no real initiating effect (only 2 type III focus) was found. This may be due to the fact that the use of rat liver S9 mix in this cell system is not yet well developed. In Syrian hamster embryo cells a transforming activity has been detected for Tris-BP and Genomoll P, but the concentrations were 10–20 times higher for the latter (Table 4). Some reports have shown that, in bacterial systems, Tris-BP is more active following metabolic activation. It is well known that hamster embryo cells possess considerable metabolic activity, and this may perhaps explain the relatively high transformation frequency found with these cells compared to that found with C3H10T1/2 cells. The negative results obtained with Tris-BP (Table 7, group 1) in the *in vivo* skin test for direct carcinogenic activity do not corroborate Van Duuren *et al.*'s results [9]. This difference is probably due to the frequency of treatment: twice weekly in our experiment and thrice weekly in the cited one. However, the results concerning the incidence of the other tumours are comparable in the two experiments. The most important information is that Tris-BP shows an initiating and not a promoting activity (Table 7, group 2), which indicates the probable induction of latent lesions in the skin cells of people who have been in contact with Tris-BP-containing nightwear. The short-term skin test (Table 6), which can detect a carcinogenic or promoting effect but not an initiating effect, was negative and therefore in agreement with the long-term skin test. Genomoll P showed no significant carcinogenic, initiating or promoting potential on the skin. Using both Tris-BP and Genomoll P as promoters, the number of lung adenomas when the mice were initiated by DMBA was lower than when they were not initiated. We have no explanation for the role of DMBA in this decrease.

In general the results reported indicate that

Tris-BP is a potential initiator for mouse epidermal cells and can thus be used for studies relative to the molecular nature of initiation. Genomoll P is less or not at all hazardous in its present use compared to Tris-BP. Nevertheless, any extension of this use needs information on human exposure and a better knowledge of its biological activities.

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