Table II—Statistical Summary of Collaborative Results of GLC Analysis of Vitamin E

Statistic	Sample[a] 1	2	3	4	5	6	7	8	9	10	11
All laboratories											
Mean	385.9	203.0	306.0	289.4	953.1	1000.5	945.5	1003.0	738.9	297.3	492.5
Reproducibility	15.1	16.0	16.3	19.4	27.2	41.2	28.5	26.4	47.6	20.0	20.4
CV, %	3.9	7.9	5.3	6.7	2.9	4.1	3.0	2.6	6.4	6.7	4.2
Repeatability	3.0	16.0	15.8	7.2	20.5	14.2	18.0	15.6	15.8	15.8	8.7
CV, %	0.8	7.9	5.2	2.5	2.2	1.4	1.9	1.6	2.1	5.3	1.8
Without Laboratory 5											
Mean	384.4	202.5	306.8	290.8	953.4	1011.0	948.5	1007.8	749.8	297.0	497.4
Reproducibility	15.1	8.4	13.6	19.4	25.7	23.4	25.8	21.4	31.0	18.6	12.6
CV, %	3.9	4.1	4.4	6.7	2.7	2.3	2.7	2.1	4.1	6.2	2.5
Repeatability	3.2	4.6	8.7	5.5	11.9	14.6	10.5	14.8	10.0	8.8	7.0
CV, %	0.8	2.3	2.8	2.6	1.3	1.4	1.1	1.5	1.3	3.0	1.4

[a] For all laboratories and samples, the mean was 601.4, the reproducibility was 27.1 with a coefficient of variation of 4.5%, and the repeatability was 14.5 with a coefficient of variation of 2.4%. When Laboratory 5 was eliminated, the mean was 604.5, the reproducibility was 20.6 with a coefficient of variation of 3.4%, and the repeatability was 9.8 with a coefficient of variation of 1.6%.

($p < 0.01$) laboratory × sample interaction. Six of the laboratories obtained consistent results for both samples. Four of the five remaining laboratories obtained results that were considerably higher for Sample 10, while one laboratory reported considerably higher results for Sample 4. Because of the significant laboratory × sample interaction, no significant difference ($p < 0.05$) was found between Samples 4 and 10.

One laboratory failed to identify properly the isomer present in Sample 10. It was learned that the procedure had not been followed correctly. Therefore, in this instance, the procedure was not at fault. All other laboratories identified the isomer present in Sample 10. The required isomer identification was properly carried out for all other samples by all laboratories.

CONCLUSION

Even with the data of the poorest performing laboratory included, coefficients of variation of 4.5% for reproducibility and 2.4% for repeatability are within the 5% required by NF. The laboratory exhibiting the poorest performance can be eliminated statistically, and the resulting coefficients of variation are 3.4 and 1.6% for reproducibility and repeatability, respectively. The reproducibility of 2.1% and the repeatability of 1.5% for the α-tocopheryl acid succinate are exceptionally gratifying since this compound is suspected of breaking down during GLC analysis and would be expected to exhibit larger coefficients of variation. The method, as collaboratively studied, appears to meet the requirements for an NF compendial method.

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Absence of Povidone-Iodine-Induced Mutagenicity in Mice and Hamsters

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Received November 3, 1977, from the *Department of Industrial Hygiene and Toxicology, BASF Aktiengesellschaft, D-6700 Ludwigshafen/Rh., Federal Republic of Germany.* Accepted for publication May 19, 1978.

Abstract □ Povidone-iodine USP was tested for mutagenicity in mice by the dominant lethal assay or micronucleus test and in Chinese hamsters by the bone marrow test. None of the three tests revealed any evidence of mutagenic effect.

Keyphrases □ Povidone-iodine—evaluated for mutagenicity in mice and hamsters □ Mutagenicity—povidone-iodine evaluated in mice and hamsters □ Anti-infectives, topical—povidone-iodine, evaluated for mutagenicity in mice and hamsters

According to Wlodkowski *et al.* (1), povidone-iodine blocked growth of the DNA polymerase-deficient *Escherichia coli* strain whereas no mutagenic effects were found with *Salmonella typhimurium* in the same (Ames) test. In the fluctuation test, povidone-iodine was mutagenic only for *S. typhimurium* T 1530 and not for *S. typhimu-*

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Table I—Testing of Povidone-Iodine in the Dominant Lethal Test on Male NMRI Mice when Applied in a Single Intraperitoneal Dose[a]

Dose	Mating Week	Females with Implantations		Total Implantations		Live Embryos		Dead Implantations		Mutagenicity Index
		Number	%	Number	Per Female	Number	Per Female	Number	Per Female	
0	1	48	80.0	548	11.4	491	10.2	57	1.2	10.4
	2	58	96.7	771	13.3	705	12.2	66	1.1	8.6
	3	58	96.7	750	12.9	690	11.9	60	1.0	8.0
	4	56	93.3	734	13.1	671	12.0	63	1.1	8.6
	5	57	95.0	737	12.9	666	11.7	71	1.2	9.6
	6	58	96.7	736	12.7	655	11.3	81	1.4	11.0
	7	58	96.7	761	13.1	705	12.2	56	1.0	7.4
	8	54	90.0	731	13.5	652	12.1	79	1.5	10.8
Control: 10 ml of distilled water/kg	1	43	71.7	516	12.0	473	11.0	43	1.0	8.3
	2	54	90.0	721	13.4	652	12.1	69	1.3	9.6
	3	57	95.0	717	12.6	646	11.3	71	1.2	9.9
	4	56	93.3	761	13.6	671	12.0	90	1.6	11.8[b]
	5	59	98.3	776	13.2	699	11.8	77	1.3	9.9
	6	58	96.7	802	13.8	716	12.3	86	1.5	10.7
	7	60	100.0	772	12.9	716	11.9	56	0.9	7.3
	8	56	93.3	779	13.9	712	12.7	67	1.2	8.6
72 mg/kg	1	33	55.0[c]	345	10.5	296	9.0	49	1.5	14.2
	2	58	96.8	772	13.3	695	12.0	77	1.3	10.0
	3	59	98.3	735	12.5	653	11.1	82	1.4	11.2
	4	58	96.7	772	13.3	716	12.3	56	1.0	7.3
	5	59	98.3	784	13.3	719	12.2	65	1.1	8.3
	6	58	96.7	811	14.0	744	12.8	67	1.2	8.3
	7	56	93.3	679	12.1	607	10.8	72	1.3	10.6[b]
	8	57	95.0	749	13.1	674	11.8	75	1.3	10.0

[a] Mating was one male to three females. [b] $p < 0.05$. [c] $p < 0.01$.

rium T 1538.

The fluctuation test showed an increase in the mutant frequency of a Valin-sensitive *E. coli* K 12 strain under the influence of povidone-iodine at an incubation temperature of 4° only and not at 37°[1].

According to investigations carried out by Speck *et al.* (2), povidone-iodine is capable of selectively altering the DNA of human diploid cells growing in culture. These investigations, and the importance of povidone-iodine as a local antiseptic, prompted the testing of povidone-iodine for mutagenicity in various animal test systems.

EXPERIMENTAL

Male and female NMRI mice[2] and male and female Chinese hamsters[3] (*Gricetulus griseus*) received standardized feed[4], and drinking water was available *ad libitum*. The animals were housed in plastic (polycarbonate) cages in air-conditioned rooms at 20 ± 2° and 55 ± 5% humidity. The day/night rhythm was 12 hr.

Povidone-iodine USP (iodine as I_3^-[1] ion complex bonded to povidone; available iodine 11.2%) dissolved in distilled water was administered intraperitoneally. The results were checked for significant differences using the χ^2 test or U test.

Dominant Lethal Assay—This assay is an indirect method of detecting induced mutations in the germ cells of male animals, because the results are found in female animals mated with (treated) males. An increase in the number of dead implantations is the main evidence of induced dominant lethal mutation. The conception rate and the average number of implantations also are used for evaluation purposes.

The test procedure used was that of Röhrborn and Vogel (3). One group of 20 male mice received 72 mg of povidone-iodine/kg (one-fifth LD_{50}).

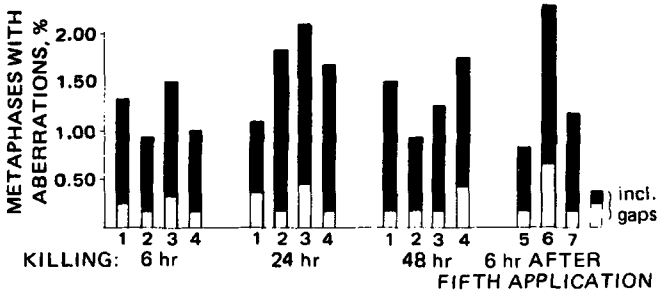

Figure 1—*Bone marrow test results in hamsters after intraperitoneal application of povidone-iodine. Key: Group 1, control; Group 2, control (sodium chloride); Group 3, 1 × 38.3 mg/kg; Group 4, 1 × 82.5 mg/kg; Group 5, control; Group 6, control (sodium chloride); and Group 7, 5 × 38.3 mg/kg.*

Table II—Testing of Povidone-Iodine in the Micronucleus Test on NMRI Mice with Double Intraperitoneal Application

Dose	0	2 × 10 ml of Distilled Water/kg	2 × 36 mg/kg
Number of animals	10	10	10
Analyzed normochromatic erythrocytes	40,000	40,000	40,000
With micronuclei, ‰	2.15	3.17	2.52
Minimum, ‰	0.12	0.12	0.15
Maximum, ‰	0.35	0.45	0.35
1 micronucleus, ‰	1.90	2.82	2.35
2 micronuclei, ‰	0.22	0.30	0.15
3 micronuclei, ‰	0.00	0.05	0.00
More than 3 micronuclei, ‰	0.02	0.00	0.02
Analyzed polychromatic erythrocytes	20,000	20,000	20,000
With micronuclei, ‰	3.70	2.95	4.30[a]
Minimum, ‰	0.20	0.20	0.20
Maximum, ‰	0.50	0.50	0.65
1 micronucleus, ‰	3.60	2.75	4.20
2 micronuclei, ‰	0.10	0.20	0.05
3 micronuclei, ‰	0.00	0.00	0.05
More than 3 micronuclei, ‰	0.00	0.00	0.00

[a] $p < 0.05$.

[1] F. Lingens, Universität Hohenheim, Institut für Mikrobiologie, Garbenstrasse 30, D-7000 Stuttgart 70, Federal Republic of Germany, personal communication.

[2] Supplied by Wiga Co., Sulzfeld, Federal Republic of Germany.

[3] Supplied by Thomae Co., Biberach, Federal Republic of Germany.

[4] Altromin R, supplied by Altromin Co., Lage/Lippe, Federal Republic of Germany; or Ssniff H, supplied by Intermast GmbH, Boeckum-Hoevel, Federal Republic of Germany.

Table III—Testing of Povidone-Iodine in the Bone Marrow Test on Chinese Hamsters with Single Intraperitoneal Application

Dose	Untreated			10 ml of Saline/kg			38.3 mg/kg			82.5 mg/kg		
Put to death after, hr	6	24	48	6	24	48	6	24	48	6	24	48
Number of animals	12	11	12	12	12	12	12	11	12	12	12	12
Analyzed metaphases	1200	1100	1200	1200	1200	1200	1200	1100	1200	1200	1200	1200
Animals with aberrant metaphases	8	8	9	7	9	6	9	8	8	6	9	9
Metaphases with aberrations												
Including gaps	16	12	18	11	22	11	18	23	15	12	20	21
Excluding gaps	3	4	2	2	2	2	4	5	2	2	2	5
Gaps (chromatide)	9	8	14	7	15	9	10	15	10	7	12	16
Gaps (isochromatide)	4	0	2	2	5	0	4	3	3	3	6	0
Breaks (chromatide)	0	2	1	1	0	2	2	2	2	1	0	2
Breaks (isochromatide)	0	0	0	0	1	0	0	0	0	0	0	0
Acentric fragments	3	1	1	0	1	0	2	3	0	1	1	3
Multiple aberrations	0	1	0	1	0	0	0	0	0	0	1	0

A second group of 20 male mice received 10 ml of distilled water/kg in a single application. The third group of 20 male mice remained untreated. One male was mated to three females.

Micronucleus Test—This test is a conventional screening method of indirectly discovering induced structural and numeric chromosome aberrations in somatic cells. Increased appearance of micronuclei in erythrocytes can be an indication of indirect mutations and also of spindle poisons.

The test procedure used was that of Boller and Schmid (4) or Schmid (5). The three groups were each comprised of five male and five female mice. One group received 36 mg of povidone-iodine/kg (one-tenth LD_{50}), and another group received 10 ml of distilled water/kg twice at an interval of 24 hr. The third group of animals remained untreated. Six hours after the final dose, the animals were sacrificed, the femurs were removed, and bone marrow smears were prepared; 4000 normochromatic and 2000 polychromatic erythrocytes were examined per animal.

Bone Marrow Test—This test was carried out in accordance with published recommendations (6) and involved 15 groups of six male and six female Chinese hamsters each. This test is used for direct analysis of induced structural and numerical chromosome aberrations in somatic cells.

Three groups of animals received a single 38.3-mg/kg (one-quarter LD_{50}) or 82.5-mg/kg (one-half LD_{50}) dose of povidone-iodine or 10 ml of saline solution/kg. Three groups remained untreated. Two other groups received five applications of 38.3 mg of povidone-iodine/kg or five 10-ml/kg applications of saline solution on 5 successive days. Another group of animals remained untreated. The animals were sacrificed 6, 24, or 48 hr after the single application or 6 hr after the fifth application; 100 metaphases were evaluated per animal.

Table IV—Testing of Povidone-Iodine in the Bone Marrow Test on Chinese Hamsters with Five Intraperitoneal Injections [a]

Dose	Untreated	5 × 10 ml of Saline/kg	5 × 38.3 mg/kg
Number of animals	12	11	11
Analyzed metaphases	1200	1100	1100
Animals with aberrant metaphases	7	8	7
Metaphases with aberrations			
Including gaps	10	25	13
Excluding gaps	2	7	2
Gaps (chromatide)	8	17	8
Gaps (isochromatide)	0	1	3
Breaks (chromatide)	1	6	2
Breaks (isochromatide)	0	0	0
Acentric fragments	0	1	0
Multiple aberrations	1	0	0

[a] Animals put to death 6 hr after the fifth application.

RESULTS

Dominant Lethal Assay (Table I)—The animals tolerated a single application of povidone-iodine without symptoms. The conception rate decreased significantly during the 1st week, but the average number of implantations and the mutagenicity index were not affected. During the other mating periods, all parameters varied in a similar manner as in the control animals.

Micronucleus Test (Table II)—The animals tolerated administration of povidone-iodine without symptoms. There was merely a slight increase in polychromatic erythrocytes containing micronuclei, but even this increase was within the normal range.

Bone Marrow Test (Tables III and IV and Fig. 1)—The animals tolerated povidone-iodine with no symptoms when administered in a single dose, but repeated application caused the animals to show signs of pain lasting for ~1 min. There was no increase in the percentage of aberrant metaphases when the substance was administered singly or repeatedly. The type of aberration was the same with treated and untreated animals.

DISCUSSION

Contrary to previous results (1, 2), povidone-iodine did not have any mutagenic effect in the dominant lethal assay, the micronucleus test, or the bone marrow test.

Because of its available iodine content, povidone-iodine has an antibacterial effect. The mutagenic effect with an *E. coli* or *S. typhimurium* strain could be due to lower sensitivity, which can result from a mutation. However, this hypothesis is unlikely since resistance does not develop following the use of povidone-iodine. Possibly, povidone-iodine also is inactivated so quickly in mammalian metabolism that genetic damage cannot result. Some genetic experts think that mutagenicity testing of microorganisms does not in any way represent ultimate testing for obtaining information, mutagenic or otherwise, and that positive *in vitro* tests cannot be used as an argument in favor of mutagenic hazard in humans when tests on mammals turn out negative.

The findings of the present animal experiments suggest that povidone-iodine has no mutagenic effects since no mutagenic effect was detected even when extremely high doses were administered.

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