Applied Organometallic Chemistry (1990) 4 231–237

0268-2605/90/030231-07$05.00

Comparative study on the tumorigenicity in mice of gallium arsenide, gallium phosphide and gallium oxide following subcutaneous and intraperitoneal injections

Akiyo Tanaka, Akira Hisanaga, Miyuki Hirata and Noburu Ishinishi
Department of Hygiene, Faculty of Medicine, Kyushu University, 3–1–1 Maidashi, Higashi-ku, Fukuoka 812, Japan

Received November 1989 *Accepted 3 March 1990*

Chronic toxicity, including tumorigenicity, of gallium arsenide (GaAs), gallium phosphide (GaP) and gallium oxide (Ga_2O_3) was studied in male ICR mice which received either a subcutaneous or an intraperitoneal injection of each material once only. The doses received either subcutaneously or intraperitoneally were 48 mg kg^{-1} as Ga or 480 mg kg^{-1} as Ga of each material suspended in 0.2 cm^3 of olive oil. The control groups received the vehicle only, either subcutaneously or intraperitoneally.

In the study using subcutaneous injections, no tumors were observed in the subcutis at the site of injection, and there was no significant difference concerning the survival periods of each group compared with the control group at the termination of the observation period.

In the study using intraperitoneal injections, the total tumor incidence in all the experimental groups, except for the GaAs (480 mg Ga kg^{-1}) group, was significantly different compared with the control group. However, all these tumors appeared to be spontaneous, rather than induced by the materials themselves. Moreover, in the GaAs (480 mg Ga kg^{-1}) and Ga_2O_3 (48 mg Ga kg^{-1}) groups, the number of survival days was significantly lower compared with the control group.

From this study, it seems that neither GaAs, GaP nor Ga_2O_3 were tumorigenic to mice when injected subcutaneously. Although it remains unclear whether the increased production of total tumors in each group following intraperitoneal injections was directly due to the tumorigenic action of GaAs, GaP or Ga_2O_3 or not, it appears that these substances produce a potential systemic toxicity in mice following intraperitoneal injections.

Keywords: Toxicity, tumorigenicity, gallium arsenide, gallium phosphide, gallium oxide

INTRODUCTION

Gallium arsenide (GaAs) is the material of choice for use in high-frequency microwave and millimeter-wave telecommunications and for use in ultrafast supercomputers.[1] With the increased incidence of the industrial use of GaAs, the question of whether the exposure of semiconductor workers to GaAs is a potential occupational health hazard or not has been gaining attention. GaAs contains the element arsenic, which is a highly toxic material suspected of being tumorigenic. Epidemiological studies have indicated a causal relationship between exposure to inorganic arsenic compounds and skin or lung cancer.[2–4] However, although inorganic arsenic compounds have been studied for tumorigenicity by various routes of administration, most results have been insufficient to demonstrate tumorigenicity in experimental animals. Recently, some data have been available concerning the tumorigenicity of some inorganic arsenic compounds, in particular $Ca_3(AsO_4)_2$, to the respiratory tract of Syrian golden hamsters following intratracheal instillations.[5,6] On the other hand, Webb *et al.*[7–9] and Goering *et al.*[10] reported the acute pulmonary toxicity of GaAs to the trachea of rats, when instilled intratracheally. In addition, Ohyama *et al.*[11] reported the chronic toxicity of GaAs when instilled intratracheally to hamsters. It is therefore important that adequate toxicity data should be acquired in order to estimate correctly the risk to semiconductor workers from exposure to GaAs or other semiconductor materials. In this study,

we attempted to assess, by means of either intraperitoneal or subcutaneous injections, the chronic toxicities, including tumorigenicity, of gallium arsenide (GaAs) and gallium phosphide (GaP), both of which are used in the semiconductor industry, and of gallium oxide (Ga_2O_3), which being a gallium compound, served as a comparison with GaAs and GaP.

EXPERIMENTAL

GaAs was obtained from Sumitomo Metal Mining Industry Co. Ltd, Japan. GaP (99.9999% pure) was obtained from Mitsuwa Chemicals, Osaka, Japan. Ga_2O_3 (99.99% pure) was obtained from Katayama Chemicals, Osaka, Japan. Olive oil was purchased from Wako Pure Chemicals, Osaka, Japan. The sample of GaAs or GaP was pulverized in an agate mortar and sieved through a 325-mesh or 200-mesh microsieve. The sizes of GaAs and GaP particles were measured with an image analyzer (Nikon Co. Ltd, Tokyo, Japan) using scanning electron microscopy (SEM).

All male ICR mice were purchased at four weeks of age from the colony of Nihon Clea, Tokyo, Japan. The mice were raised under conventional conditions at 22–25 °C for four weeks until the beginning of the experiment. The mice were housed ten per plastic cage, fed a commercial diet (CE-2 pellets, Nihon Clea, Tokyo, Japan) and given water *ad libitum*.

Three hundred mice (36.0–37.5 g at eight weeks of age) were divided into 12 groups of 20 mice plus two control groups of 30 mice. The control groups received the vehicle only, either subcutaneously or intraperitoneally. Of the remaining 12 groups, six groups received subcutaneous injections in the intrascapular region of either 48 mg Ga kg^{-1} of GaAs, 480 mg Ga kg^{-1} of GaAs, 48 mg Ga kg^{-1} of GaP, 480 mg Ga kg^{-1} of GaP, 48 mg Ga kg^{-1} of Ga_2O_3 or 480 mg Ga kg^{-1} of Ga_2O_3 suspended in 0.2 cm^3 of olive oil, once only, at the age of eight weeks. The doses were chosen in accordance with those of Webb *et al.*[7–9] The other six groups received intraperitoneal injections of the same quantities and substances.

All mice were observed during a period of 18 months and weighed once every two weeks. Except for a few animals lost through postmortem changes or cannibalism, all of those which died during the period or were killed after the observation period were autopsied, and the subcutaneous tissue and principal visceral organs were fixed in 10% formalin solution. For the purposes of histopathological examinations, sections were prepared by conventional methods and stained with hematoxylin and eosin. Selected sections were stained with periodic acid Schiff (PAS) or van Gieson.

The survival curve of each group examined was assessed by the Kaplan–Meier method[12] and a generalized Wilcoxon test[13] or chi-square test was used for statistical comparison of tumor incidence in each group.

RESULTS AND DISCUSSION

Analysis of particle size

The mean count diameter for GaAs and GaP particles was $6.13 \pm 7.43\,\mu m$ and $6.31 \pm 10.00\,\mu m$, respectively. The majority of Ga_2O_3 particles were less than $1.0\,\mu m$ in diameter according to analysis by SEM.

The study using subcutaneous injections

The materials administered by subcutaneous injections did not significantly effect the cumulative body-weight gain of the mice. The survival patterns of each experimental group are shown in Fig. 1. The mean numbers of survival days were as follows:

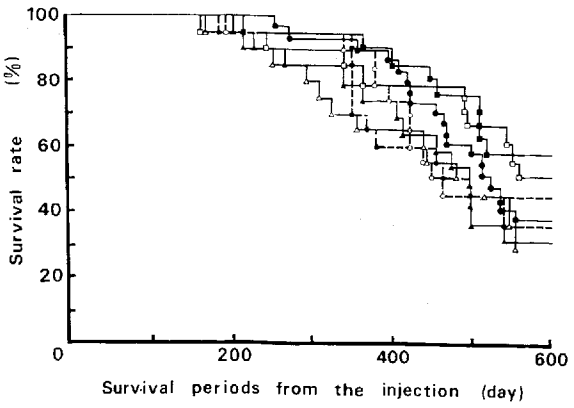

Figure 1 Change in the survival rate (%) of each experimental group following the subcutaneous injection. GaAs: 48 mg Ga kg^{-1} –●—●–; 480 mg Ga kg^{-1} –○—○–. GaP: 48 mg Ga kg^{-1} –△—△–; 480 mg Ga kg^{-1} –▲—▲–. Ga_2O_3: 48 mg Ga kg^{-1} –■—■–; 480 mg Ga kg^{-1} –□—□–. Control, –●—●–.

Table 1 Tumor incidence in mice which received subcutaneous injections of GaAs, GaP or Ga_2O_3.

Group (dose, mg Ga Kg^{-1})	Sex	No. of mice examined[a]	No. of tumor-bearing mice						Total tumor incidence (%)
			Subcutis						
			is[b]	nis[c]	Lung	Liver	HPS[d]	Total	
GaAs (48)	M	19/20	0	1	4	2	4	9	47
GaAs (480)	M	20/20	0	1	3	0	3	6	30
GaP (48)	M	20/20	0	0	1	0	1	2	10
GaP (480)	M	20/20	0	2	5	0	4	10	50
Ga_2O_3 (48)	M	20/20	0	2	4	0	1	7	35
Ga_2O_3 (480)	M	20/20	0	0	6	0	7	11	55
Control	M	28/30	0	1	6	2	7	15	54

[a] Mice were observed for 18 months from start of the experiment. [b] is, injection site only. [c] nis, not including injection site. [d] HPS, Hematopoietic system.

GaAs groups: 453 days at 48 mg Ga kg^{-1}
466 days at 480 mg Ga kg^{-1}
GaP groups: 436 days at 48 mg Ga kg^{-1}
452 days at 480 mg Ga kg^{-1}
Ga_2O_3 groups: 508 days at 48 mg Ga kg^{-1}
494 days at 480 mg Ga kg^{-1}
Control group: 489 days

During the first six months, none of the mice died. Although higher mortality rates were observed in the GaAs (48 mg Ga kg^{-1}) and GaP (48 mg Ga kg^{-1}) groups during the first year, the difference in the survival rates between each group and the control group was not significantly different, according to the Kaplan–Meier method.[12]

Tumors of the subcutis, the lung, the liver and the hematopoietic system are shown in Table 1. No subcutaneous tumors developed at the injection site in any group. However, particles of GaAs, GaP or Ga_2O_3 were seen to be deposited in the subcutaneous tissue in almost all the mice and a proliferation of fibrous connective tissue was observed at the surrounding deposition area of each particle (Fig. 2). Other neoplasms were seen elsewhere in the subcutis, the lung, the liver and the hematopoietic system. The total tumor incidence rates were:

GaAs groups: 47% at 48 mg Ga kg^{-1}
30% at 480 mg Ga kg^{-1}
GaP groups: 10% at 48 mg Ga kg^{-1}
50% at 480 mg Ga kg^{-1}
Ga_2O_3 groups: 35% at 48 mg Ga kg^{-1}
55% at 480 mg Ga kg^{-1}
Control group: 54%

The incidences of total tumors in the groups given GaAs, GaP and Ga_2O_3 were not significantly greater than that in the control group according to the chi-square test or a generalized Wilcoxon test.[13]

The study using intraperitoneal injections

No marked differences were noted in the change of body weights between the GaAs, GaP or Ga_2O_3 groups and the control group. Although the weights of the GaAs (480 mg Ga kg^{-1}) and GaP (480 mg Ga kg^{-1}) groups increased relatively more slowly than the weight of the control group, this was not statistically significant.

The survival patterns of each group are shown in Fig. 3. The mean numbers of survival days were:

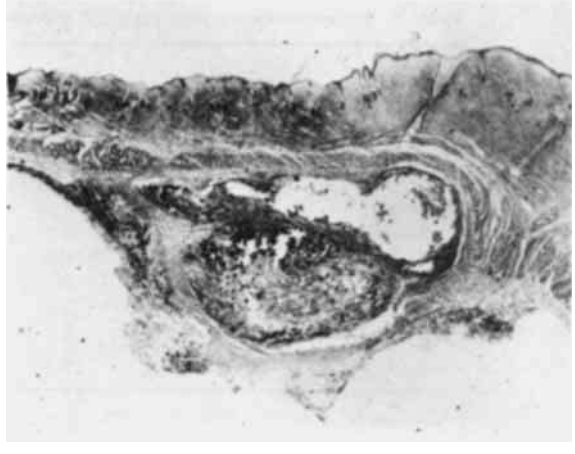

Figure 2 Deposition of GaAs particles in the subcutaneous tissue of the back following subcutaneous injections. H.E. stain, magnification ×18.

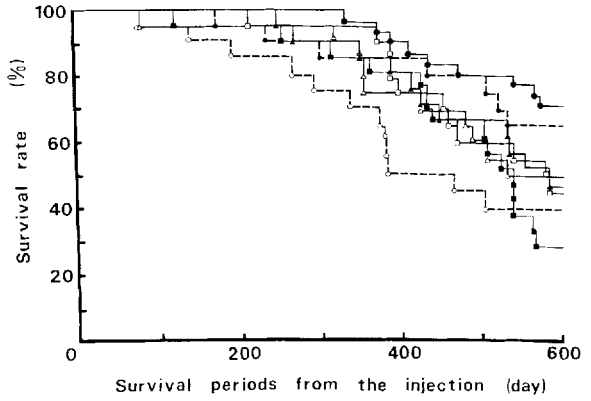

Figure 3 Change in the survival rate (%) of each experimental group following the intraperitoneal injection. GaAs: 48 mg Ga kg^{-1} –●–●–; 480 mg Ga kg^{-1} –○–○–. GaP: 48 mg Ga kg^{-1} –△–△–; 480 mg Ga kg^{-1} –▲–▲–. Ga_2O_3: 48 mg Ga kg^{-1} –■–■–; 480 mg Ga kg^{-1} –□–□–. Control, –●–●–.

GaAs groups:	507 at 48 mg Ga kg^{-1}
	422 at 480 mg Ga kg^{-1}
GaP groups:	488 at 48 mg Ga kg^{-1}
	505 at 480 mg Ga kg^{-1}
Ga_2O_3 groups:	484 at 48 mg Ga kg^{-1}
	507 at 480 mg Ga kg^{-1}
Control group:	553 days

During the first three months, none of the mice died. The difference in the survival rates between the GaAs (480 mg Ga kg^{-1}) or Ga_2O_3 (48 mg Ga kg^{-1}) groups and the control group was significant, but no significant difference between the other groups and the control group was found by the Kaplan–Meier method.[12]

No tumors of the peritoneum were observed in any group. Tumors were manifested in the subcutis, the spleen, the lung, the liver and the hematopoietic system and these are shown in Table 2. The total tumor incidence rates were:

GaAs groups:	56% at 48 mg Ga kg^{-1}
	17% at 480 mg Ga kg^{-1}
GaP groups:	50% at 48 mg Ga kg^{-1}
	50% at 480 mg Ga kg^{-1}
Ga_2O_3groups:	45% at 48 mg Ga kg^{-1}
	70% at 480 mg Ga kg^{-1}
Control group:	38%

The difference in the rates of total tumor production between the Ga_2O_3 (480 mg Ga kg^{-1}) group and the control group was significant, but between the other groups and the control group it was not significant by the chi-square test. However, in all the experimental groups, except the GaAs (480 mg Ga kg^{-1}) group, the total tumor incidence was significantly different compared with the control group, according to a generalized Wilcoxon test.[13]

Besides tumor formation, depositions of GaAs, GaP or Ga_2O_3 particles were seen, focally involving the serous membrane of the diaphragm, the peritoneum or the mesentery in almost all the mice, and a proliferation of the fibrous connective tissue or a moderate cellular inflammatory response surrounding the deposition site of each particle was observed in some of the mice (Fig. 4). Moreover, depositions of each particle were commonly seen in the capsule of the liver,

Table 2 Tumor incidence in mice which received intraperitoneal injections of GaAs, GaP or Ga_2O_3

Group (dose, mg Ga Kg^{-1})	Sex	No. of mice examined[a]	No. of tumor-bearing mice						Total tumor incidence (%)
			Subcutis	Spleen	Lung	Liver	HPS[b]	Total	
GaAs (48)	M	18/20	2	0	3	3	3	10	56[d]
GaAs (480)	M	18/20	0	1	0	0	3	3	17
GaP (48)	M	20/20	0	1	4	1	4	10	50[d]
GaP (480)	M	20/20	0	0	5	1	4	10	50[e]
Ga_2O_3 (48)	M	20/20	3	0	4	1	2	9	45[d]
Ga_2O_3 (480)	M	20/20	3	0	5	2	6	14	70[e,f]
Control	M	29/30	0	0	8	0	3	11	38

[a] Mice were observed for 18 months from the start of the experiment. [b] Hematopoietic system. [c] Significantly different from the control group (chi-square test, $P<0.05$). [d] Significantly different from the control group (generalized Wilcoxon test, $P<0.01$). [e] Significantly different from the control group (generalized Wilcoxon test, $P<0.05$). [f] Significantly different from the control group (generalized Wilcoxon test, $P<0.001$).

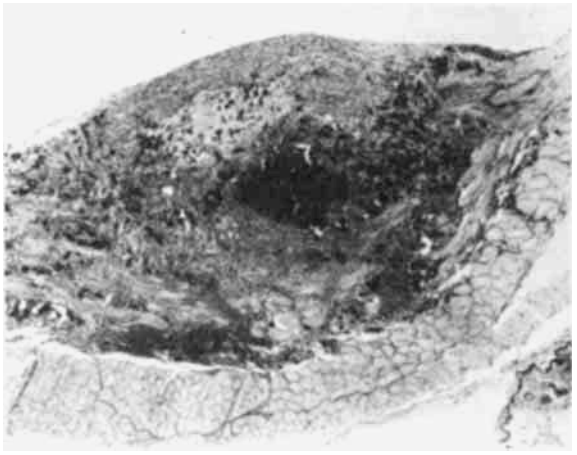

Figure 4 Deposition of GaAs particles in the serious membrane following intraperitoneal injections. H.E. strain, magnification ×23.

the kidney, the spleen or the testis, but rarely in the parenchyma of those organs or the lymph nodes.

Toxicological aspects

Some studies have indicated that GaAs particles, when instilled intratracheally, produce acute or chronic pulmonary toxicity or systemic toxicity.[7-11] In our present study using intraperitoneal injections, the total tumor incidence in all the experimental groups, except the GaAs (480 mg Ga kg^{-1}) group, was significantly different compared with the control group, and in the GaAs (480 mg Ga kg^{-1}) and Ga_2O_3 (48 mg Ga kg^{-1}) groups, the number of survival days was significantly lower than in the control group. However, in our present study using subcutaneous injections, there were neither marked findings concerning total tumor incidence nor marked changes in survival rates in any of the experimental groups compared with the control group. Therefore, it is clear that GaAs, GaP and Ga_2O_3 produce systemic injury when administered intraperitoneally but not when administered subcutaneously. Furthermore, only a few particles which were likely to be carried through the lymph stream were observed in various organs, and there was no extensive hyperplasia of the fibrous connective tissue surrounding the deposition areas of particles in the back following subcutaneous injections. A greater number of depositions of particles were found in many organs following intraperitoneal injections than following subcutaneous injections. It could be that the differences in total tumor incidence and shorter survival rates were caused by such depositions of particles.

Concerning acute toxicity, Webb *et al.*[7] mentioned that GaAs administered orally and intratracheally to rats caused systemic arsenic intoxication and GaAs instilled intratracheally showed more toxicity than when administered orally. Webb *et al.*[8] showed that GaAs instilled intratracheally to rats caused severe lung damage, but less intensive pathological changes to the lung than Ga_2O_3. In addition, Webb *et al.*[9] reported that intratracheal administration of smaller GaAs particles to rats produced more severe pulmonary pathogenic lesions and more rapid signs of systemic arsenic toxicity than they had found in their previous study using larger fractions of GaAs particles.[7,8] In this study, we injected large fractions of GaAs and other substances into mice, but since the manifestation of toxicity may be closely related to the particle size, perhaps it would have been more appropriate for us to have used small particles in our investigations of toxicity or tumorigenicity.

Goering *et al.*[10] found inflammatory responses in the lung, swelling of the proximal tubule mitochondria in the kidney and inhibition of δ-aminolevulinic acid dehydratase (ALAD) in the blood, kidney, and liver following a single intratracheal instillation of GaAs to rats. On the other hand, Webb *et al*,[7,8] Yamauchi *et al.*[14] and Rosner and Carter[15] all reported that GaAs was only slightly soluble when administered orally, intratracheally or intraperitoneally to rats or hamsters, and that inorganic arsenic compounds are released partially from GaAs when administered by various routes. Nevertheless, when the doses of GaAs given to mice were relatively large, equal to those used by Webb *et al.*,[7-9] neither acute toxicity nor a marked increase in tumors of the lung and skin, each which is usually closely related to exposure to inorganic arsenic compounds, was observed. It seems that inorganic arsenic compounds, if released from GaAs, show little systemic toxic effect in mice.

Although epidemiological studies have revealed a causal relationship between exposure to inorganic arsenic compounds and skin or lung cancer,[2-4] and although recent studies using laboratory animals have produced some positive evidence concerning tumorigenicity of inorganic arsenic compounds to the respiratory organs,[4] it is likely that in our study the lower survival rates

and increased total tumor incidences in both GaAs groups receiving intraperitoneal administrations were directly caused by the GaAs particles themselves rather than by the inorganic arsenic compounds derived from GaAs.

When we try to evaluate the tumorigenicity of chemical substances to humans, long-term inhalation studies are in fact appropriate exposure models for animal experiments, these being analogous to human exposure. Nevertheless it is recognized as worthwhile to ascertain if the tumorigenicity of semiconductor materials, including GaAs, can be verified by use of several administration routes, such as intraperitoneal injections and subcutaneous injections, even though these cannot be directly extrapolated to humans. Regarding total tumor incidence following intraperitoneal injections, there was a significant increase compared with the control group only in the Ga_2O_3 (480 mg Ga kg^{-1}) group as judged by the χ^2 test. However, the time of tumor appearance was significantly earlier in all the groups except for the GaAs (480 mg Ga kg^{-1}) group, as determined by a generalized Wilcoxon test. Concerning spontaneous tumor incidence in ICR strain mice, Yamate *et al.*[16] reported that tumors of the liver (incidence rate: 35.9%), the lung (41.0%), and the hematopoietic system (7.7%) were observed most commonly in male mice, with a few tumors being seen in the brain, the skin, the harderian gland, the prepuce and the testis. The results of our present study are practically consistent with those findings regarding the site of tumor appearance and the rate of tumor incidence, although a higher tumor incidence was observed in the hematopoietic system. Such an increased incidence of tumors in mice treated intraperitoneally seems to be due to the chronic physical action of the materials concerned rather than to their chemical properties.

To date, there are no available data to clarify whether there is a relationship between exposure to semiconductor materials and the incidence of human cancer. As for epidemiological studies, Sorahan *et al.*[17] reported that there was no increasing mortality or morbidity from all cancers in a cohort of semiconductor workers, although three cases of melanoma of the skin were found (expected number: 0.68). However, this was not a significant increase. In addition, Pastides *et al.*[18] reported an increased rate of spontaneous abortion and general illness symptoms among workers in semiconductor factories. It is a fact that GaAs particles cause pulmonary toxicity when instilled intratracheally to laboratory animals, and that inorganic arsenic compounds are released from GaAs partially. However, we cannot ignore the possible adverse health effects of exposure to other semiconductor materials, and far more research is needed.

CONCLUSIONS

Our results show that neither GaAs, GaP nor Ga_2O_3 are tumorigenic, nor do they produce toxicity to mice when injected subcutaneously. However, these substances produce a potential systemic toxicity following the intraperitoneal injections, which might enhance the time of tumor manifestation, although it remains unclear whether such action is really directly due to the tumorigenic action of GaAs, GaP and Ga_2O_3 or not.

Acknowledgements We are grateful to Miss K Miller for editorial assistance. This study was supported by Grant 61480165 from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Robinson, A L *Science*, 1983, 219: 275
2. International Agency for Research on Cancer, Some metals and metallic compounds. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, vol 23, IARC, Lyon, 1980, pp 39–141
3. International Agency for Research on Cancer, Chemicals, industrial processes and industries associated with cancer in humans. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, supplement 4, IARC, Lyon, 1982, pp 50–52
4. International Agency for Research or Cancer, Overall evaluations of carcinogenicity, *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, supplement 7, IARC, Lyon, 1987, pp 100–106
5. Pershagen, G and Björklund, N-E *Cancer Lett.*, 1985, 27: 99
6 Yamamoto, A, Hisanaga, A and Ishinishi, N *Int. J. Cancer*, 1987, 40: 220
7 Webb, D R, Sipes, I G and Carter, D E *Toxicol. Appl. Pharmacol.*, 1984, 76: 96
8 Webb, D R, Wilson, S E and Carter, D E *Toxicol. Appl. Pharmacol.*, 1986, 82: 405
9 Webb, D R, Wilson, S E and Carter, D E *Am. Ind. Hyg. Assoc. J.*, 1987, 48: 660
10 Goering, P L, Maronpot, R R and Fowler, B *Toxicol. Appl. Pharmacol.*, 1988, 92: 179

11 Ohyama, S, Ishinishi, N, Hisanaga, A and Yamamoto, A *Appl. Organomet. Chem.*, 1988, 2: 333
12 Kaplan, E L, and Meier, P *J. Am. Stat. Assoc.*, 1977, 53: 457
13 Gehan, E A *Biometrika*, 1965, 52: 203
14 Yamauchi, H, Takahashi, K and Yamamura, Y *Toxicology*, 1986, 40: 237
15 Rosner, M H and Carter, D E *Fund. Appl. Toxicol.*, 1987, 9: 730
16 Yamate, J, Tajima, M and Kudow, S *Jpn. J. Vet. Sci.*, 1986, 48: 273
17 Sorahan, T, Waterhouse, J A H, McKiernan, M J and Aston, R H R *Br. J. Ind. Med.*, 1985, 42: 546
18 Pastides, H, Calabrese, E J, Hosmer, D W and Harris, D R *J. Occup. Med.*, 1988, 30: 543