Published in final edited form as:

Int J Toxicol. 2010 ; 29(3): 291–296. doi:10.1177/1091581810362804.

Arsenic Exposure In Utero and Nonepidermal Proliferative Response in Adulthood in Tg.AC Mice

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Abstract

To expand our knowledge on the transplacental carcinogenic potential of inorganic arsenic, pregnant Tg.AC mice received drinking water with 0, 42.5, or 85 ppm arsenite from gestation day 8 to 18. After birth, groups (n = 25) of offspring received topical 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) (2 µg twice a week) for 36 weeks and were killed; nonskin tumors were assessed. Arsenic increased adrenal cortical adenomas (ACAs; 25%-29%) compared with control (0%) independent of TPA in all male groups. Arsenic increased urinary bladder (UB) hyperplasia in males, but only with TPA. Arsenic induced ACAs in all female groups (control 0%; arsenic 17%-26%). Arsenic-treated females had UB hyperplasia in most groups (control 0%; arsenic 26%-32%), with 2 UB papillomas. All arsenic-treated females had uterine hyperplasia (26%-40%; control 4%) independent of TPA, and 3 had uterine tumors. Thus, arsenic in utero rapidly induces ACAs and uterine and UB preneoplasias in Tg.AC mice, showing transplacental carcinogenic potential in yet another strain of mice.

Keywords

arsenic; carcinogenesis; transplacental; rodent; adrenal tumors

Arsenic shows remarkable early-life stage carcinogenic activity in humans and mice.^{1–4} Emerging data indicate that arsenic exposure in the perinatal period can cause liver and lung cancers in humans.^{1,2} In mice, in utero arsenic exposure acts as a complete transplacental carcinogen in organs such as the liver, lung, ovary, and adrenal (for review, see Waalkes et al³). Fetal arsenic exposure can predispose mice to skin cancers later in life induced by other agents.⁴ Specifically, in Tg.AC mice, fetal arsenic exposure via maternal drinking water renders the animals highly susceptible to treatment with the skin tumor promoter/nongenotoxic carcinogen 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) when applied topically long after arsenic exposure has ended, forming a clear excess of squamous cell carcinoma,⁴ a skin tumor associated with arsenic exposure in humans.⁵ This indicates that arsenic may have set into place molecular events in the fetus that can, under the right

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Declaration of Conflicting Interests

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

conditions, be stimulated to become cancers during adulthood and could be argued as a toxicological version of fetal basis of adult disease susceptibility.

The skin is an important target site for arsenic carcinogenesis in humans,^{5,6} and the epidermal carcinogenesis results from the prior Tg.AC transplacental arsenic study⁴ were therefore given priority for publication. However, other tumors and significant, potential preneoplastic lesions occurred in these Tg.AC mice that were not reported in the initial article.⁴ Because some of the unreported lesions in Tg.AC mice exposed during the fetal life stage involve tissues consistent with human target sites (ie, urinary bladder [UB]) or with target sites repeatedly found in mouse transplacental arsenic studies (ie, uterus and adrenal cortex),^{3,7-10} these results are being reported to fortify the existing database with tumor data from an additional mouse strain. This report describes the nonskin proliferative lesions induced by prenatal arsenic exposure with or without postnatal TPA application in Tg.AC mice.

Methods

Animals and Treatment

Animal care was provided in accordance with the US Public Health Policy on the Care and Use of Animals as defined in the *Guide to the Care and Use of Animals* (NIH Publication 86-23). Mice were housed under conditions of controlled temperature, humidity, and light cycle. Homozygous Tg.AC mice were obtained from Taconic Farms (Germantown, NY). Mice were supplied 5L-79 rat-mouse (18%) feed (Ralston Purina, St Louis, Mo) ad libitum.

For nonskin tumors, 30 timed primigravid mice were randomly divided into groups of 10 and given drinking water with sodium arsenite (NaAsO_2 ; Sigma, St Louis, MO) at 0 (control), 42.5 ppm arsenite (low), or 85 ppm arsenite (high) ad libitum from day 8 to 18 of gestation. These doses of arsenic did not affect maternal water consumption. After birth, offspring were weaned at 4 weeks and randomly assigned to groups (initial $n = 25$) of males or females based on maternal exposure and then exposed to TPA (2 μg per 0.1 mL of acetone twice a week to a freshly shaved area of dorsal skin; Sigma) or vehicle (acetone) for 36 weeks. Dam body weights (daily) and water consumption (gestation days 7, 8, 14, 18) were recorded. Offspring weights were recorded weekly from birth. Mice were checked daily and sacrificed when significant clinical signs developed or at 40 weeks.

Tumor Assessment

A complete necropsy was performed. The following tissues were routinely taken: liver, lung, kidney, spleen, adrenal, UB, ovary, uterus, oviduct, testes, skin, and grossly abnormal tissues. Tissues were fixed in buffered formalin, sectioned, and stained with hematoxylin and eosin. Pathological assessments were performed such that there was no prior knowledge of the treatment.

Data Analysis

Data are expressed as tumor incidence or mean $\pm$ standard error of the mean for body weights. Tumor incidence comes from 23 to 25 mice per treatment group originally derived

at random from 10 litters per treatment group. The decrease in some groups represents animals found dead and considered too autolytic for appropriate histological evaluation. Incidence data were compared by 1-sided Fisher's exact test. Epidermal cancer lesions from these mice have been reported previously.⁴ Body weights were compared by Dunnett's multiple comparison test after analysis of variance. $P = .05$ was considered significant in all cases.

Results

Pregnant Tg.AC mice received arsenic in the drinking water from gestation days 8 to 18 at doses that result in fetal blood inorganic arsenic levels similar to humans with arsenicosis and that induce nonskin tumors in the offspring of other mouse strains.³ The doses were well tolerated by the dam, fetus, and neonate based on maternal water consumption, maternal and neonatal body weights, and weight gains of the offspring.⁴ After birth and weaning (4 weeks), the offspring received topical TPA through adulthood to 40 weeks of age (see Methods for details). The treatment with TPA, both alone and combined with arsenic, did not affect body weight at any time point in either males or females (Table 1). However, because TPA by itself and especially in combination with prior fetal arsenic exposure produced a high rate of skin tumors⁴ requiring euthanasia of the animals, all groups receiving TPA showed smaller group sizes remaining, especially after 25 to 30 weeks of age (21-26 weeks of TPA treatment; see Table 1).

In male Tg.AC offspring, proliferative lesions associated with prenatal arsenic exposure included adrenal cortical adenoma (ACA) and UB hyperplasia (Table 2). Adrenal cortical adenomas were consistently increased by fetal arsenic exposure in males from 0% in controls to between 25% and 29% in animals receiving arsenic in utero. Adrenal cortical tumor induction by fetal arsenic exposure appeared independent of the addition of TPA treatment during adulthood, which itself did not induce adrenal cortical tumors. Furthermore, ACA formation did not follow a clear arsenic dose response for unknown reasons. The early loss of animals to observation (see Table 1) because of the need to kill mice with multiple skin cancers in the TPA treatment groups did not appear to hinder arsenic-induced ACA formation in these groups (Table 2). In contrast, UB hyperplasia was not observed in controls and was relatively uncommon with TPA alone (8.7%, not significantly increased compared with control, $P = .234$). With prenatal exposure to arsenic alone there were nonsignificant increases in UB hyperplasia that approached significance ($P = .0546$) in the group receiving 85 ppm arsenic alone (17%). However, when combined with postnatal TPA, both doses of fetal arsenic produced significant ($P < .05$) increases in UB hyperplasia, although this was not related to the arsenic dose.

In female Tg.AC offspring, proliferative lesions associated with prenatal arsenic exposure included adrenal cortical tumors and UB and uterine hyperplasia (Table 3). Arsenic treatment resulted in induction of ACAs in most groups (arsenic 42.5 ppm, arsenic 42.5 ppm + TPA, and arsenic 85 ppm + TPA) whereas the incidence of ACA in the group receiving 85 ppm arsenic alone approached statistical significance (17%; $P = .0502$) compared with control (0%). Like males, the TPA-alone female groups had no adrenal cortical tumors and TPA appeared to have limited or no impact on the ability of arsenic to induce ACAs. The

incidence of ACAs was not related to the arsenic dose. In addition, arsenic-treated females had UB hyperplasia in most groups (arsenic 42.5 ppm; arsenic 42.5 ppm + TPA; arsenic 85 ppm + TPA), with 2 UB papillomas, 1 in a group receiving arsenic alone and 1 in a group receiving arsenic plus TPA. The group receiving 85 ppm arsenic alone did not show UB hyperplasia although when 85 ppm arsenic exposure in utero was followed by postnatal TPA, it induced a significant rate of UB hyperplasia (32%).

Proliferative lesions of the uterus also occurred with prenatal arsenic in Tg.AC mice (Table 3). This included uterine hyperplasia after fetal arsenic exposure, the incidence of which was between 26% and 40% with arsenic regardless of other treatments and was uncommon in control (4%) or with TPA alone (4%). There were also 3 tumors of the uterus in arsenic-alone groups: 1 adenoma and 2 leiomyomas.

For perspective, pooled proliferative lesions induced by fetal arsenic exposure in male or female Tg.AC mice were examined (Table 4) and revealed several important facts. First, a preponderance of adrenal cortical tumors, UB hyperplasias, and uterine hyperplasias are associated with prenatal arsenic exposure in a highly significant fashion. Second, although not reaching statistical significance, tumors of both the UB and uterus only occurred in the animals that had been exposed to arsenic in utero.

Several tumors occurred that were incidental and not associated with any treatment (Table 5). This included lung tumors, leukemia/lymphoma, and jaw odontoma (common in Tg.AC mice).

Two ovarian adenomas occurred in animals that received prenatal arsenic alone (1 each in the 42.5 ppm and 85 ppm groups). These tumors are noteworthy because transplacental arsenic has been repeatedly associated with ovarian tumors in other mouse strains (C3H and CD1) in several studies⁷⁻⁹ in which the animals reached a much greater age (90 weeks) than in the present work (40 weeks).

Discussion

Inorganic arsenic is a known human carcinogen that affects millions of people worldwide.^{5,6} Recent evidence indicates that perinatal exposure to inorganic arsenic is carcinogenic later in life in humans,^{1,2} and multiple studies show that inorganic arsenic has clear transplacental carcinogenic activity in mice.⁷⁻¹⁰ As a transplacental carcinogen in mice, arsenic targets multiple organs, producing tumors in adulthood in such tissues as the lung, liver, adrenal cortex, and uterus and often producing hyperplasia of uterus or UB.⁷⁻¹⁰ We also find that fetal inorganic arsenic exposure via maternal drinking water in Tg.AC mice, which are genetically susceptible to skin cancer, predisposes them to development of advanced skin carcinoma when exposed to the skin tumor promoter/carcinogen TPA later in life.⁴ However, only skin tumors were reported in this work,⁴ although arsenic could have affected other sites. Indeed, here we show that fetal arsenic did cause multiple proliferative lesions in nonskin sites from these mice and, clearly, fetal exposure to arsenic induced a significant response in Tg.AC mice when they became adults.

The tumor response was primarily in the adrenal cortex where adenomas were induced by fetal inorganic arsenic exposure in both males and females in a fashion largely independent of TPA. The response was not related to arsenic dose, although the study ended much earlier in life (40 weeks of age) than in prior transplacental work with C3H mice (74 weeks or 104 weeks)^{7,8} or with CD1 mice (90 weeks).^{9,10} Prior work has also shown ACA in both males and females,⁷⁻¹⁰ and 1 study has seen a fetal arsenic dose-response relation in induction of adrenal adenoma in adulthood, with a maximal incidence of 91 %.⁷ The reasons for a lack of a dose response in ACA are unclear but may be due to the relatively brief nature of this study compared with prior transplacental work with arsenic.⁷⁻¹⁰ The early assessment (40 weeks of age) may have obscured final incidences of these nonfatal tumors. The addition of TPA, which required early skin tumor-related removal of mice from this study,⁴ starting at about 25 weeks (see Table 1), had little or no impact on eventual ACA incidence. This means these adrenal tumors formed very early after in utero exposure to arsenic. The exact mechanism of this adrenal tumor formation is not known, but fetal arsenic may alter genetic programming in the mouse adrenal, facilitating enhanced steroid-linked gene expression in the adrenal later in life.¹¹ This could have the potential to affect tumors locally or at other target tissues of arsenic carcinogenesis.

Arsenic was also able to induce a significant incidence of UB hyperplasia, which in females occurred in almost all arsenic-exposed groups (Table 3) but in males occurred in mice exposed to prenatal arsenic plus postnatal TPA (Table 2). Two papilloma of the UB also occurred in female Tg.AC mice treated with arsenic in utero. In rats, oral exposure to dimethylarsinic acid (DMA^V) in drinking water for up to 2 years induces UB tumors.^{12,13} The tumors induced were papilloma and transitional cell carcinoma (TCC). The UB is a key target tissue of arsenic in humans, and TCC is concordant with the tumor type most often found in exposed human populations.^{5,6} Also, when DMA^V was mixed with food, UB tumors were induced in female rats but not males.¹⁴ The UB tumors are again papillomas and TCC.¹⁴ Potential preneoplastic lesions (UB urothelial hyperplasia) are also increased in male rats.¹⁴ The reasons for these gender-based differences in sensitivity are unknown. The study by Arnold et al¹⁴ is quite comprehensive and presents critical confirmatory evidence that oral DMA^V can induce UB cancer in rats. Arnold et al¹⁴ also studied male and female mice treated with DMA^V but did not observe UB tumors. The basis of the species-related difference in susceptibility to arsenic-induced UB carcinogenesis is undefined, but mice may be insensitive to UB cancer induced by arsenicals alone. However, exposure to arsenicals plus other agents does stimulate formation of UB proliferative lesions in mice or rats.^{9,10,15,16} For instance, postnatal exposure to diethylstilbestrol (DES) or tamoxifen stimulates UB hyperplasia, papilloma, or TCC formation in mice exposed to arsenic via maternal drinking water.^{9,10} Furthermore, in a study where rats were initiated with a complex mixture of organic carcinogens followed by DMA^V in the drinking water for 24 weeks, the rats given the carcinogen mix plus DMA^V developed UB tumors not seen with the arsenical alone.¹⁶ These data indicate that DMA^V, a biomethylation product of inorganic arsenic that would be expected in the urine, can promote UB carcinogenesis.¹⁶ In another promotion-type study, rats were given oral N-butyl-N-(4-hydroxybutyl)nitrosamine as an initiator and then DMA^V for up to 32 weeks.¹⁶ The combined organic carcinogen and biomethylation product of arsenical treatment increased UB papilloma and carcinoma, whereas DMA^V alone had no

effect in the UB.¹⁶ Thus, accumulating data indicate that both inorganic arsenic and biomethylation products of inorganic arsenic can cause or promote/initiate UB tumors in rodents.^{9,10,12–16} The results of Devesa et al¹⁷ show that maternal exposure to inorganic arsenic during gestation in mice results in significant amounts of mono- and di-biomethylation products in fetal blood and tissues. The ability to induce UB proliferative lesions in rodents is important because the UB is an important target site in humans exposed to arsenic.^{5,6} The results of the present study, although primarily showing early arsenic-induced UB proliferative lesions, add to this accumulating data and need to be placed in the context of how early the study ended (40 weeks of age) relative to other studies with much longer observation periods.^{12–14}

The uterus was also a target organ of arsenic in this study, and here all female prenatal arsenic groups showed uterine hyperplasias independent of TPA (Table 3). Three uterine tumors were found in arsenic-exposed groups, whereas none were found in control or TPA-treated groups. Uterine hyperplasia, along with occasional uterine tumors, has been seen in prior transplacental studies with arsenic in C3H mice,^{7,8} and an increase in uterine tumors has been observed in CD1 mice exposed to arsenic in utero.⁹ Clearly, estrogen actions at estrogen receptors (ERs) are an causal factor in uterine cancers.¹⁸ In this regard, fetal arsenic exposure in mice stimulates genes downstream of ER- α in the neonatal uterus (ie, *CYP2A4*, *pS2* and *lactoferrin*) and greatly enhances uterine expression of these genes after exposure to the synthetic estrogen DES.⁹ Uterine proliferative lesions induced by fetal arsenic exposure and promoted by postnatal DES show a remarkable overexpression of ER- α .⁹ This also occurs in other target sites in mice, such as the liver, where estrogen signaling is greatly enhanced in hepatocellular carcinoma induced by fetal arsenic exposure.¹⁹ ER- α is elevated in livers from persons exposed to high levels of environmental inorganic arsenic,¹⁹ and the liver is now a suspected target site of arsenic carcinogenesis in humans.⁶ Thus, at least for some tissues, arsenic may act, in part, through an estrogen-related mechanism.^{9,19}

This study showed that transplacental arsenic exposure in Tg.AC mice rapidly produced adrenal tumors and hyperplasias of the UB and uterus in the offspring as they became adults. These sites are consistent with previous findings and lend support to prior work showing that arsenic has transplacental oncogenic potential in C3H and CD1 mice.^{7–10}

Acknowledgments

We thank Dr L. Keefer and Dr W. Qu for critical review of this paper. The content of this publication does not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Funding

The author(s) disclosed receipt of the following financial support for the research and/or authorship of this article: This research was supported, in part, by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. This research was funded, in part, with Federal funds from the NCI under contract HHSN261200800001E.

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Table 1.

Time on Test and Body Weight in Tg.AC Mice Exposed to Arsenic In Utero and Postnatal TPA^a

	Mice Remaining, n						Body Weight, Mean ± SEM		
	Wk 15 ^b	Wk 20	Wk 25	Wk 30	Wk 35	Wk 40	Wk 5	Wk 35	
Males									
Control	24	22	20	20	20	19	28.6 ± 1.9	36.5 ± 2.6	
TPA ^c alone	18	16	11 ^e	9 ^e	6 ^e	6 ^e	27.2 ± 1.5	36.5 ± 3.9	
As (42.5) ^d	24	23	23	23	21	20	28.4 ± 1.6	36.0 ± 2.8	
As (85)	24	24	22	20	20	19	27.0 ± 1.5	37.1 ± 5.3	
As (42.5) + TPA	19	15	12 ^e	7 ^e	6 ^e	1 ^e	26.9 ± 1.6	34.2 ± 3.6	
As (85) + TPA	24	22	18	11 ^e	7 ^e	5 ^e	26.5 ± 1.9	34.0 ± 4.5	
Females									
Control	25	24	23	22	20	18	22.8 ± 1.4	31.2 ± 4.9	
TPA alone	22	18	17	13 ^e	9 ^e	7 ^e	24.1 ± 1.3	31.9 ± 3.5	
As (42.5)	24	22	22	19	18	18	22.5 ± 1.9	29.9 ± 3.	
As (85)	22	22	22	21	17	15	23.2 ± 2.3	30.9 ± 4.5	
As (42.5) + TPA	19	10 ^e	9 ^e	7 ^e	4 ^e	0 ^e	21.6 ± 1.6	27.2 ± 4.8	
As (85) + TPA	21	20	13 ^e	6 ^e	6 ^e	6 ^e	22.2 ± 2.5	30.7 ± 1.8	

As, arsenic; SEM, standard error of the mean; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.^aSee Methods for treatment details.^bWeeks refer to weeks of age.^cTPA was applied to the shaved back from the time of weaning (4 weeks) to 40 weeks of age, when all animals were killed and assessed for tumor burden.^dThe maternal level of arsenic (in ppm) in the drinking water is given in parentheses and was provided to the pregnant female between gestation day 8 and 18.^eSignificant difference ($P < .05$) from control.

Table 2.Tumors and Proliferative Lesions in Male Tg.AC Mice Exposed to Arsenic In Utero and Postnatal TPA^a

Treatment Group	n	Adrenal Adenoma, n (%)	Bladder Hyperplasia, n (%)
Control	24	0 (0)	0 (0)
TPA alone	23	0 (0)	2 (8.7)
As (42.5)	24	7 (29) ^b	3 (13)
As (85)	24	7 (29) ^b	4 (17) ^c
As (42.5) + TPA	25	7 (28) ^b	7 (28) ^b
As (85) + TPA	24	6 (25) ^b	5 (21) ^b

As, arsenic; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

^aSee Methods for treatment details. Under treatment group, the maternal level of arsenic (in ppm) in the drinking water is given in parentheses and was provided to the pregnant female between gestation day 8 and 18. TPA was applied to the shaved back from the time of weaning (4 weeks) to 40 weeks of age when all animals were killed and assessed for tumor burden. Skin cancers resulting from this study have already been reported.⁴ Data are shown as number of lesion-bearing animals (% total).

^bSignificant difference ($P < .05$) from control by 1-sided Fisher's exact test.

^cApproached significance ($P = .0546$ compared with control by 1-sided Fisher's exact test).

Table 3.

Tumors and Proliferative Lesions in Female Tg.AC Mice Exposed to Arsenic In Utero and Postnatal TPA ^a

Treatment Group	n	Adrenal Adenoma, n (%)	Urinary Bladder, n (%)		Uterus, n (%)		
			Hyperplasia	Papilloma	Hyperplasia	Adenoma	Leiomyoma
Control	25	0 (0)	0 (0)	0 (0)	1 (4.0)	0 (0)	0 (0)
TPA alone	24	0 (0)	0 (0)	0 (0)	1 (4.0)	0 (0)	0 (0)
As (42.5)	23	4 (17) ^b	6 (26) ^b	1 (4.3) ^c	9 (39) ^b	1 (4.3)	1 (4.3)
As (85)	24	4 (17) ^d	3 (13)	0 (0)	8 (33) ^b	0 (0)	1 (4.2)
As (42.5) + TPA	23	6 (26) ^b	7 (30) ^b	1 (4.3) ^c	6 (26) ^b	0 (0)	0 (0)
As (85) + TPA	25	5 (20) ^b	8 (32) ^b	0 (0)	10 (40) ^b	0 (0)	0 (0)

As, arsenic; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

^aSee Table 2 for details.

^bSignificant difference ($P < .05$) from control.

^cBoth urinary bladder papillomas occurred in mice that also had separate areas of hyperplasia.

^dApproached statistical significance from control ($P = .0502$).

Pooled Proliferative Lesions in Male or Female Tg.AC Mice Exposed to Either Level of Arsenic Regardless of Other Treatment and Compared With Control^a

Pooled Treatment Group	Adrenal Adenoma, n (%)	Bladder, n (%)		Uterus, n (%)	
		Hyperplasia	Papilloma	Hyperplasia	Tumors
Control	0/49 (0)	0/49 (0)	0/49 (0)	1/25 (4)	0/25 (0)
TPA alone	0/47 (0)	2/47 (4)	0/47 (0)	1/24 (4)	0/24 (0)
All As ^b	46/192 (24) ^c	52/192 (27) ^c	2/192 (1)	33/95 (35) ^c	3/95 (3)
As alone	22/95 (23) ^c	16/95 (17) ^c	1/95 (1)	17/47(36) ^c	3/47 (6)
As + TPA	24/97 (25) ^c	20/97 (21) ^c	1/97 (1)	16/48 (33) ^c	0/48 (0)

As, arsenic; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

^aSee Table 2 for details.

^bIncluded animals receiving TPA as well as arsenic.

^cSignificant difference ($P < .05$) from control.

Common Incidental Tumors Unrelated to Treatment in Male or Female Tg.AC Mice Exposed to Arsenic In Utero and Postnatal TPA^a

Table 5.

Group	n	Lung			Leukemia/Lymphoma	Jaw Odontoma
		Adenoma	Carcinoma			
Males						
Control	24	2	1	3		0
TPA alone	23	0	0	5		2
As (42.5)	24	2	0	2		0
As (85)	24	0	0	1		2
As (42.5) + TPA	25	0	0	2		0
As (85) + TPA	24	0	3	1		2
Females						
Control	24	0	0	1		3
TPA alone	23	1	0	2		2
As (42.5)	24	0	0	2		0
As (85)	24	1	2	4		3
As (42.5) + TPA	25	0	0	5		0
As (85) + TPA	24	0	0	3		3

As, arsenic; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.^aSee Table 2 for details.