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# Relevance of the mouse skin initiation–promotion model for the classification of carcinogenic substances encountered at the workplace

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ABSTRACT

The Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission of the Deutsche Forschungsgemeinschaft) evaluates chemical substances using scientific criteria to prevent adverse effects on health at the work place. As part of this task there is a need to evaluate tumor promoting activity of chemicals (enhancement of formation of squamous cell carcinomas via premalignant papillomas) obtained from two-stage initiation/promotion experiments using the mouse skin model. In the present communication we address this issue by comparing responses seen in mouse skin with those in humans. We conclude that tumor promotional effects seen in such animal models be carefully analyzed on a case by case basis. Substances that elicit a rather non-specific effect that is restricted to the high dose range are considered to be irrelevant to humans and thus do not require classification as carcinogens. In contrast, substances that might have both a mode of action and a potency similar to the specific effects seen with TPA (12-*O*-tetradecanoylphorbol-13-acetate), the prototype tumor promoter in mouse skin, which triggers receptor-mediated signal cascades in the very low dose range, have to be classified in a category for carcinogens.



## 1. Current situation and issue

The "classical" standardized (Schmidt and Hecker, 1989; Filler et al., 2007) two-stage carcinogenesis experiment (synonym: initiation–promotion model) in dorsal mouse skin provides valuable mechanistic information on chemically provoked skin cancer development. In this standardized procedure, a carcinogen such as 7,12-dimethylbenz[*a*]anthracene (DMBA) is applied for tumor initiation – as a single dose – followed by repeated treatment with a tumor promoter, such as the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which is not or only weakly carcinogenic (Boutwell, 1964; Hecker, 1987; Slaga, 1983). Repeated doses of TPA following a single application of DMBA induce papillomas on the dorsal skin of treated mice. Some of these papillomas (about 5%) progress to invasively growing squamous cell carcinomas (DiGiovanni, 1992).

During the 1980s and 1990s, further substances were identified that – depending on the tumor-promoting activity of the specific substance – also induced varying numbers of papillomas as well as a small percentage of malignant degeneration, when tested under the two-stage initiation–promotion regimen on mouse skin.

However, development of papillomas in experimental studies is essentially limited to the mouse species, whereas only minimal or even no papillomas are induced in other rodent species or in minipigs (Stenback, 1980; Slaga, 1983). Likewise, human skin does not react to chemical application or irritation in the form of skin papillomas. As premalignant lesions (carcinomas in situ; actinic keratosis) and squamous cell carcinomas of the human skin do not develop via precursors of papillomas, there is no direct analogy between papillomas of the mouse skin and actinic keratosis (DiGiovanni, 1992).

A considerable number of workplace agents are known which are able to produce tumors in mouse skin and enhance the incidence of skin cancer in exposed humans, These include arsenic, polycyclic hydrocarbons (e.g., from coal tar products), ultraviolet radiation and ionizing radiation (e.g.. see Gawkrodger, 2004).

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These agents, however, are "complete" carcinogens with a genotoxic mechanism of action and are, for this very reason, not in the center of interest of the present paper.

Rather, we want to address the question, whether non-genotoxic substances that have been shown to promote tumors in the two-stage carcinogenesis experiment with dorsal mouse skin should be classified in one of the categories for carcinogenic occupational substances.

## 2. The classical DMBA/TPA tumor-promotion model in mice

### 2.1. Quantitative aspects of DMBA/TPA tumor promotion in the skin

The dose and time response of TPA promotion was investigated in numerous experimental studies. Lutz et al. (1996) analyzed papilloma development on the dorsal mouse skin at a constant DMBA dose and variable TPA doses. Very low doses of DMBA (2 nmol; twice weekly) were applied in combination with TPA. The authors observed saturation of the papilloma response in the high TPA dose range. A 50% papilloma response was reached after ~7 weeks at 10 nmol/application and after ~8 weeks at 3 nmol. A papilloma prevalence of 100% was observed only slightly later. However, at 1 nmol TPA, the mean papilloma induction time was ~25 weeks and at 0.3 nmol it was ~50 weeks. Papilloma were no longer induced in mice treated with 0.1 nmol. The authors interpreted this as a "no effect threshold at low dose". The response differed when the DMBA dose varied. The mean papilloma induction time was ~10 weeks at 3 and 10 nmol DMBA, ~12 weeks at 1 nmol and ~18 weeks at 0.3 nmol (plus 2.5 nmol TPA in each case). Based on the mean papilloma induction time, TPA, but not DMBA, showed a marked loss of effect with decreasing doses (Lutz et al., 1996).

Further studies have demonstrated that repeated application of TPA is necessary to induce papillomas. If the interval between treatments is markedly extended (the standard experiment uses 2 applications per week), no promoting effect is observed. Moreover, repeated treatment for at least about 10 weeks is required; TPA treatment for only 5 weeks did not lead to papilloma development (Burns et al., 1983; Kopp-Schneider and Portier, 1992). A maximum TPA-induced papilloma response was observed after treatment for about 20 weeks. In the standard experiment, the papilloma response did not increase further if treatment was continued; instead, the rate of papillomas continuously decreased to a value of about 20% beyond 20 weeks of treatment irrespective of whether TPA was applied further or not (Kopp-Schneider and Portier, 1992). This observation shows that papillomas induced by DMBA/TPA on the dorsal mouse skin have a strong tendency to spontaneous regression. However, there are marked differences among strains. Whereas most papillomas disappear in C57BL mice, ~50% of papillomas convert to carcinomas in FVB mice (Hennings et al., 1993).

### 2.2. Strain and species differences in sensitivity to phorbol esters

The available studies indicate a distinct relationship between the phorbol ester response and the genetic background of the mouse strain used. SENCAR mice are most sensitive followed by NMRI, CD-1 and C57BL strains (Marks and Fürstenberger, 1995). In an earlier study (Shubik, 1950), rats, hamsters, rabbits and guinea pigs were completely resistant to tumor promotion induced by phorbol esters. In studies by Goerttler and coworkers (1980, 1984), weak effects of TPA were observed in rats and Syrian golden hamsters. The overall results of the various studies show that the strong tumor promotion induced by TPA in mice is not observed in other rodent species or is effective only to a considerably reduced extent. Minipigs were not susceptible to a papilloma reaction either (Stenback, 1980; Slaga, 1983).

Phorbol esters induce inflammatory reactions and hyperplasia of the skin. In mice, the severity of the inflammatory reaction correlates at least partially with the papilloma-promoting activity (Hecker, 1963). For two tested phorbol esters, studies with volunteers showed that skin irritation was lower by about a factor of 10 as compared with mice (Hickey et al., 1981). This suggests that humans are a species that is less sensitive to the irritant effects induced by phorbol esters.

### 2.3. Papilloma-carcinoma progression in the DMBA/TPA tumor-promotion model in mice

After initiation and promotion for several weeks, the mouse skin model produces a wide variety of skin changes: cauliflower-like, pedunculated or flatly spread papillomas and skin areas that are not prominent, but only thickened (hyperplastic), that appear glassy in some cases and are often inflamed. Many of these changes are reversible even when repeated TPA application is continued (see above). However, presumably about 20% of persistent papillomas spontaneously develop into malignant, invasively growing squamous cell carcinomas. In NMRI mice, about 4–5% of all papillomas undergo malignant conversion to carcinomas (Fürstenberger and Kopp-Schneider, 1995). The incidence of carcinomas and the time of their occurrence depend on the DMBA and TPA doses; the lower one of the two selected risk factors is, the lower the likelihood of carcinoma development and the later the occurrence of carcinomas (Fürstenberger and Kopp-Schneider, 1995; Burns et al., 1983). However, it is not possible to increase the final number of carcinomas by further (beyond about week 15) or higher TPA doses (Fürstenberger and Kopp-Schneider, 1995; Burns et al., 1983). An increase in the frequency of malignant conversion to carcinomas can be induced by application of a genotoxic carcinogen like N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) (Hennings et al., 1983).

In recent years, comprehensive studies have been carried out to clarify why certain papillomas (high-risk papillomas) progress to squamous cell carcinomas (SCCs), whereas others regress (low-risk papillomas). Furthermore, the attempt was made to define biomarkers for the differentiation of the two forms (Darwiche et al., 2007; Glick et al., 2007; Hennings et al., 1983; Brown et al., 1998; Ridd et al., 2006). Transgenic mouse models, among others, were used for this purpose (Ridd et al., 2006; Ferreira et al., 2009). According to the available results, the majority of papillomas that do not progress to SCCs in spite of continued TPA application seem to originate from initiated interfollicular stem cells (Brown et al., 1998; Glick et al., 2007; Darwiche et al., 2007).

## 3. Evaluation of other compounds with promoting effects on the dorsal mouse skin in the two-stage initiation–promotion model

A large number of substances have tumor-promoting activity in the two-stage initiation–promotion model in dorsal mouse skin. The chemical structure of various tumor promoters is very heterogeneous, as are the assumed primary mechanism of action and the potency of the different substances. Table 1 shows some of the known active compounds (DiGiovanni, 1992).

Skin irritation is a common characteristic of the listed substances (see below). At certain doses, strong tumor promoters, such as TPA, induce a high number of papillomas, but only a relatively small percentage of them progress to carcinomas. However, weak tumor promoters, such as mezerein, induce only relatively few papillomas, but a far higher percentage of them progress to