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# A Wistar Rat Strain Prone to Spontaneous Liver Tumor Development: Implications for Carcinogenic Risk Assessment

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**The European Union legislation considers nongenotoxic substances that only cause liver tumors in certain sensitive strains of mice as *raising no concern for man.* The EU legislation, however, also clearly stipulates that cases where the only available tumor data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence are relevant arguments which exclude a concern for man. We have analyzed the spontaneous liver tumor incidence in Wistar rats and in B6C3F$_1$ and C57Bl mice used in carcinogenicity trials at the BASF facility in Ludwigshafen, Germany, over the past 15 years and compared the spontaneous liver tumor incidence in BASF Wistar rats with those observed in rat strains employed in major European contract research organizations (CROs). The results of these analyses indicate that the incidence of spontaneous liver tumors in the BASF Wistar rat strain is very high, similar to that seen in the B6C3F$_1$ mouse and much higher than that seen in the C57Bl mouse and other commonly used strains of rat. The analyses also revealed signs of increasing variability and liver tumor drift in several rat strains. Moreover, the incidence of spontaneous preneoplastic liver cell foci was far higher in the BASF Wistar rat strain than reported for other rat strains in the literature. The analyses provide relevant arguments which exclude a concern for man for nongenotoxic chemicals that only tested positive for liver tumors in this sensitive rat strain.** 

***Key Words*: spontaneous liver tumor; rat; mouse; carcinogens; EU classification; EU legislation.**

## INTRODUCTION

The current legislation in the European Union (Table 1) categorizes substances as *known to be carcinogenic to man (category 1)* if there is sufficient epidemiological evidence to establish a causal association between human exposure to a substance and development of cancer. Substances are categorized *as if they are carcinogenic to man (category 2)* if a substance induces positive carcinogenicity results in two animal species. However, such animal carcinogens may be categorized as *causing concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment (category 3)* if

- carcinogenic effects were noted only at very high dose levels exceeding the maximal tolerated dose,
- there was appearance of tumors, especially at high dose levels, only in particular organs of certain species known to be susceptible to a high spontaneous tumor formation,
- there was appearance of tumors only at the site of application in very sensitive test systems (e.g., ip or sc application of certain locally active compounds), and the particular target is not relevant to man,
- there was lack of genotoxicity in short-term tests *in vivo* and *in vitro*,
- there is a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g., hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation),
- there is a species-specific mechanism of tumor formation (e.g., by specific metabolic pathways) irrelevant for man.

An animal carcinogen should not be classified in any of these categories if the mechanism of experimental tumor formation is clearly identified, with good evidence that this process cannot be extrapolated to man, or if the only available tumor data are liver tumors in certain sensitive strains of mice.

Nongenotoxic substances that only cause liver tumors in certain sensitive strains of mice raise no concern for man. However, nongenotoxic substances that only cause liver tumors in rats are currently classified as *causing concern for man owing to possible*

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**TABLE 1**
**Classification of Carcinogens in the European Union[a]**

| Carcinogens | Definition and classification criteria | Risk phrase |
|---|---|---|
| Category 1 | *Substances known to be carcinogenic to man.*<br>There is sufficient epidemiological evidence to establish a causal association between human exposure to a substance and the development of cancer. | Toxic (T)<br>May cause cancer (R45) |
| Category 2 | *Substances which should be regarded as if they are carcinogenic to man.*<br>There is sufficient evidence from animal studies to provide a strong presumption that human exposure to a substance may result in the development of cancer:<br>• either positive results in two animal species or<br>• clear positive evidence in one species, together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumors, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association. | |
| Category 3 | *Substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.*<br>Arguments listed below are relevant which reduce the significance of experimental tumor induction in view of possible human exposure:<br>• carcinogenic effects only at very high dose levels exceeding the "maximal tolerated dose,"<br>• appearance of tumors, especially at high dose levels, only in particular organs of certain species known to be susceptible to a high spontaneous tumor formation,<br>• appearance of tumors, only at the site of application, in very sensitive test systems (e.g., ip or sc application of certain locally active compounds), if the particular target is not relevant to man,<br>• lack of genotoxicity in short-term tests *in vivo* and *in vitro*,<br>• existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g., hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation),<br>• existence of a species-specific mechanism of tumor formation (e.g., by specific metabolic pathways) irrelevant for man. | Harmful (Xn)<br>Danger of irreversible effects (R40) |
| No classification required | Arguments listed below are relevant which exclude a concern for man:<br>• a substance should not be classified in any of the categories if the mechanism of experimental tumor formation is clearly identified, with good evidence that this process cannot be extrapolated to man,<br>• if the only available tumor data are liver tumors in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories,<br>• particular attention should be paid to cases where the only available tumor data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence. | |

[a] Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the 18th time Council Directive 67/548/EEC on the approximation of the laws, regulations, and administrative provisions relating to the classification, packaging and labelling of dangerous substances, *Official Journal L 110, 04/05/1993, pp. 0020–0021.*

*carcinogenic effects* (category 3 carcinogens). The EU legislation, however, also clearly stipulates that cases where the only available tumor data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence are relevant arguments which exclude a concern for man. By implication, the induction of liver tumors by nongenotoxic substances in a rat strain with a sensitivity to spontaneous liver tumor formation similar to that seen in sensitive strains of mice should raise no concern for man.

BASF has performed carcinogenicity studies in rats and mice for approximately two decades. An initial analysis of the spontaneous liver tumor incidence in the rat strain employed in our facility (Chbb: THOM (SPF) Wistar) indicated a rather high occurrence of these tumors. As the occurrence of spontaneous tumors has implications for the risk assessment of chemicals tested, we have:

- analyzed the spontaneous liver tumor incidence in Chbb: THOM (SPF) Wistar rats and B6C3F1 and C57Bl mice used in carcinogenicity trials at the BASF facility in Ludwigshafen, Germany, over the past 15 years;
- compared the spontaneous liver tumor incidence in BASF Wistar rats with those observed in rat strains

employed in major European contract research organizations (CROs), i.e., in HanWistar, Sprague-Dawley, Fischer 344 (F344), and OFA CD rats;

- analyzed the frequency of liver tumor induction in carcinogenicity trials with nongenotoxic substances in the rat and mouse strains used by BASF and by the U.S. National Toxicology Program (NTP) Carcinogen Bioassay Program.

## MATERIALS AND METHODS

### *Investigated Rat Strains*

*Wistar rats.* This rat strain is used as both a toxicology model and a general, all purpose, outbred model for use in biomedical research. Its body weight averages less than Sprague-Dawley rats yet it exhibits comparable reproductive performance. The original stock was obtained from the Wistar Institute (Philadelphia, PA). The following substrains were analyzed for the incidence of spontaneous liver tumors: the Hanlbm : WIST rat, hereafter referred to as HanWistar rat, a cesarian-derived stock from a Wistar colony maintained at the Hannover Institute in Germany (data provided by a CRO), and the Chbb: THOM (SPF) Wistar rat used by BASF (referred to as BASF Wistar rat strain) in carcinogenicity trials over the past 20 years.

*Sprague-Dawley (SD) rats.* This rat strain is a widely accepted, general purpose research model used in virtually all disciplines of biomedical research including pharmacology and toxicology. The strain originates from Sprague-Dawley, Inc., and is maintained as an outbred colony. Data on the CD substrain, submitted by six different CROs, were analyzed.

*Fischer 344 (F344) rats.* This rat strain is commonly used for cancer research, toxicology, and ageing studies. The Fischer 344 is the inbred model of choice for the U.S. National Toxicology Program Carcinogen Bioassay Program and the U.S. National Institute on Ageing. The origin of the strain is as follows: to NIH in 1951 from Heston, to Heston in 1949 from Curtis, Columbia University Institute for Cancer Research. Data submitted by a CRO were analyzed.

*OFA SD rats.* This rat strain is of medium size with a rapid growth rate, docile, easy to work with, and that reproduces well. The original stock was composed in 1925 by Robert Worthingdon Dawley, and Carworth Farms obtained it in 1955 and renamed it CFE (Carworth Farms Elias). Data submitted by a CRO were analyzed.

### *Investigated Mouse Strains*

The B6C3F1 mouse strain is used by BASF and NTP, and the C57Bl strain is used by BASF. The data base of BASF was analyzed.

### *Analysis of Susceptibility to Nongenotoxic Liver Carcinogens*

The susceptibility of F344 rats and B6C3F$_1$ mice to nongenotoxic liver oncogens was compared using data published by the U.S. National Toxicology Program Carcinogen Bioassay Program. The frequency of liver tumor induction in carcinogenicity trials with nongenotoxic substances in the rat and mouse strains used by BASF was compared using the data collected by BASF over the past 15 years.

## RESULTS

### *Spontaneous Liver Tumor Incidence in Rats (Table 2)*

The highest mean liver tumor incidence (adenomas and carcinomas combined) in males was observed in the BASF Wistar rat (10.82%) with a range of 0 to 32% in carcinogenicity trials. Both liver adenoma and liver carcinoma incidence were highest in the male BASF Wistar rat (adenomas: 6.58% with a range of 0 to 30%; carcinomas: 4.24%, with a range of 0–10%). The lowest mean liver tumor incidence in males was observed in the OFA SD rat (0.3%) with a range of 0 to 1% in carcinogenicity trials; liver carcinoma formation was not observed.

The highest liver tumor incidence in females was observed in the BASF Wistar rat (3.60%) and the Sprague-Dawley rat (3.59%) used by CRO No. 8 with ranges of 0–20 and 0–13%, respectively. The lowest liver tumor incidence in females was observed in the OFA SD rat (0.3%) with a range of 0 to 1% in carcinogenicity trials; as in males, liver carcinoma formation was not observed.

The incidence of spontaneous liver tumors in the BASF Wistar rat strain is also higher than in rat strains used by laboratories that have published their data. Bomhard *et al.* (1986) reported the occurrence of 0 benign and 2 malignant liver tumors in 959 untreated male Wistar rats and 2 benign and 0 malignant liver tumors in 956 untreated females, i.e., the spontaneous liver tumor incidence was well below 1%. Chandra *et al.* (1992) reported an incidence for liver adenomas of 2.0% in male and 0.83% in female untreated Sprague-Dawley rats (2-year studies with 1340 males and 1329 females). Similar values have been reported for Sprague-Dawley rats by others (McMartin *et al.*, 1992).

### *Spontaneous Liver Tumor Incidence in Rats in Recent Trials (Table 3)*

The differences in liver tumor susceptibility between the various rat strains became even more apparent when recently conducted carcinogenicity trials (initiated in the nineties) were analyzed. The highest mean liver tumor incidence in males was observed in the BASF Wistar rat (15.43%) with a range of 5 to 24%.

**TABLE 2**
**Incidence of Spontaneous Liver Tumors in Various Rat Strains**

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Rat strain | Adenomas | Carcinomas | Combined | Adenomas | Carcinomas | Combined |
| OFA SD (CRO 1) | | | | | | |
| Mean | 0.31% | <0.31% | 0.31% | 0.31% | <0.31% | 0.31% |
| Range | 0–1% | 0% | 0–1% | 0–1% | 0% | 0–1% |
| *n* | 320 | 320 | 320 | 320 | 320 | 320 |
| F344 (CRO 2) | | | | | | |
| Mean | 2.47% | 0.21% | 2.68% | 1.72% | 0.14% | 1.86% |
| Range | 0–11% | 0–2% | 0–13% | 0–8% | 0–2% | 0–8% |
| *n* | 1455 | 1455 | 1455 | 1455 | 1455 | 1455 |
| Han Wistar (CRO 3) | | | | | | |
| Mean | 1.74% | 0.33% | 2.07% | 2.85% | 0.48% | 3.33% |
| Range | 0–6% | 0–2% | 0–6% | 0–10% | 0–2% | 0 –12% |
| *n* | 2130 | 2130 | 2130 | 2103 | 2103 | 2103 |
| SD (CRO 4) | | | | | | |
| Mean | 1.82% | 1.27% | 3.09% | 0.36% | <0.18% | 0.36% |
| Range | 0–24% | 0–4% | 0–28% | 0–8% | 0% | 0–8% |
| *n* | 550 | 550 | 550 | 550 | 550 | 550 |
| SD (CRO 5) | | | | | | |
| Mean | 2.81% | 2.61% | 5.42% | 2.02% | 0.20% | 2.22% |
| Range | 0–8% | 0–6% | 0–8% | 0–6% | 0–2% | 0–6% |
| *n* | 498 | 498 | 498 | 494 | 494 | 494 |
| SD (CRO 6) | | | | | | |
| Mean | 1.71% | 0.14% | 1.84% | 0.48% | <0.07% | 0.48% |
| Range | 0–4% | 0–1% | 0–5% | 0–2% | 0% | 0–2% |
| *n* | 1466 | 1466 | 1466 | 1467 | 1467 | 1467 |
| SD (CRO 7) | | | | | | |
| Mean | 2.15% | 1.16% | 3.31% | 1.87% | 0.08% | 1.95% |
| Range | 0–8% | 0–4% | 0–8% | 0–6% | 0–2% | 0–6% |
| *n* | 1116 | 1116 | 1116 | 1280 | 1280 | 1280 |
| SD (CRO 8) | | | | | | |
| Mean | 3.15% | 2.07% | 5.22% | 2.94% | 0.65% | 3.59% |
| Range | 0–8% | 0–7% | 0–13% | 0–10% | 0–3% | 0–13% |
| *n* | 920 | 920 | 920 | 918 | 918 | 918 |
| SD (CRO 2) | | | | | | |
| Mean | 2.44% | 1.89% | 4.33% | 1.10% | <0.07% | 1.10% |
| Range | 0–10% | 0–7% | 0–16% | 0–5% | 0% | 0–5% |
| *n* | 1478 | 1478 | 1478 | 1460 | 1460 | 1460 |
| BASF Wistar | | | | | | |
| Mean | 6.58% | 4.24% | 10.82% | 2.97% | 0.63% | 3.60% |
| Range | 0–30% | 0–10% | 0–32% | 0–20% | 0–5% | 0–20% |
| *n* | 1580 | 1580 | 1580 | 1580 | 1580 | 1580 |

The mean liver carcinoma incidence was also highest in the male BASF Wistar rat (5.71% with a range of 0 to 10%). The male Sprague-Dawley rat used by CRO No. 2 was second with a mean liver tumor incidence of 9.14% (range 2–16%) and a liver carcinoma incidence of 4.57% (range 0–7%).

The highest liver tumor incidence in females was also observed in the BASF Wistar strain (7.43%) with a range of 5 to 15%, followed by the female Han Wistar rat with a mean liver tumor incidence of 6.27% (range 1–12%). The incidence of liver carcinomas in females was highest in the Sprague-Dawley rat used by CRO No. 8: 1.32% with a range of 0 to 3% in carcinogenicity trials.

The data also indicated that the susceptibility to liver tumor formation of Sprague-Dawley and Wistar rats was higher than average in recent trials, which strongly suggested that increasing variability in liver tumor incidence over time had occurred in these strains.

### *Variability in Liver Tumor Incidence in Rat Strains over Time (Table 4)*

In trials initiated in the early eighties, both genders of the HanWistar rat rarely developed spontaneous liver tumors. In trials initiated in the nineties, the HanWistar rat had become far more susceptible to spontaneous liver tumor formation.

**TABLE 3**
**Spontaneous Liver Tumor Incidence in Various Strains of Rats in Recently Conducted Carcinogenicity Trials**

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Starting Year of trial | Adenomas | Carcinomas | Combined | Adenomas | Carcinomas | Combined |
| | | | BASF Wistar rats | | | |
| 1993–1998 | | | | | | |
| Mean | 9.71% | 5.71% | 15.43% | 7.14% | 0.29% | 7.43% |
| Range | 0–22% | 0–10% | 5–24% | 4–15% | 0–2% | 5–15% |
| *n* | 350 | 350 | 350 | 350 | 350 | 350 |
| | | | Sprague-Dawley rats (CRO 2) | | | |
| 1993 | | | | | | |
| Mean | 4.57% | 4.57% | 9.14% | 3.43% | <0.57% | 3.43% |
| Range | 2–10% | 0–7% | 2–16% | 2–5% | 0% | 2–5% |
| *n* | 175 | 175 | 175 | 175 | 175 | 175 |
| | | | Sprague-Dawley rats (CRO 8) | | | |
| 1992–1994 | | | | | | |
| Mean | 4.47% | 2.89% | 7.37% | 3.43% | 1.32% | 4.75% |
| Range | 1–8% | 0–7% | 2–14% | 0–10% | 0–3% | 0–13 % |
| *n* | 380 | 380 | 380 | 379 | 379 | 379 |
| | | | HanWistar rats (CRO 3) | | | |
| 1991-1993 | | | | | | |
| Mean | 3.24% | <0.3% | 3.24% | 5.96% | 0.31% | 6.27% |
| Range | 2–12% | 0% | 2–12% | 1–10% | 0–2% | 1–12% |
| *n* | 340 | 340 | 340 | 319 | 319 | 319 |
| | | | F344 rats (NTP)[a] | | | |
| 1990–1997 | | | | | | |
| Mean | 2.29% | 0.67% | 2.81% | 0.59% | 0.07% | 0.67% |
| Range | 0–10% | 0–6% | 0–10% | 0–6% | 0–2% | 0–6% |
| *n* | 1352 | 1352 | 1352 | 1351 | 1351 | 1351 |
| | | | F344 rats (CRO 2) | | | |
| 1993–1996 | | | | | | |
| Mean | 1.85% | <0.37% | 1.85% | 2.96% | <0.37% | 2.96% |
| Range | 0–4% | 0% | 0–4% | 0–8% | 0% | 0–8% |
| *n* | 270 | 270 | 270 | 270 | 270 | 270 |
| | | | OFA SD rats (CRO 1)[b] | | | |
| Mean | 0.31% | <0.31% | 0.31% | 0.31% | <0.31% | 0.31% |
| Range | 0–1% | 0% | 0–1% | 0–1% | 0% | 0–1% |
| *n* | 320 | 320 | 320 | 320 | 320 | 320 |

[a] Hasemann *et al.* (1998).
[b] Recently finalized studies.

In trials initiated in 1984–1985, spontaneous liver tumors were also rare in the *female* BASF Wistar rat. *Males* were susceptible to liver carcinoma formation, the incidence ranging from 0 to 8%. In trials conducted in the nineties both genders of BASF Wistar rats had become far more susceptible to spontaneous liver tumor formation.

In trials initiated in the mid-eighties, the Sprague-Dawley rat was susceptible to spontaneous liver tumor formation (data for two CROs (Nos. 2 and 8 ) are shown).

In trials initiated in the nineties, the Sprague-Dawley rat had developed a far higher susceptibility to spontaneous formation of liver tumors.

The F344 strain of rat showed no signs of liver tumor drift. The incidence of liver adenomas and carcinomas ranges from 0 to 8 and 0 to 2% in males and females, respectively. The combined liver tumor incidence ranges from 0 to 13 and 0 to 8% in males and females, respectively. The OFA rat showed a very low liver tumor incidence in recent studies.

### *Liver Tumor Susceptibility of B6C3F$_1$ Mice (Table 5)*

Male B6C3F1 mice used by BASF were found to be very susceptible to spontaneous liver tumor formation. The incidence of liver adenomas and carcinomas ranges from 0 to 14 and 4 to 22%, respectively. Female B6C3F1 mice are less susceptible to spontaneous liver tumor formation, the incidence of adenomas and carcinomas ranging from 0 to 10% and 0 to 6%, respectively. The combined incidence of liver tumors ranges from 14 to 36% and 0 to 12% in males and females, respectively.

**TABLE 4**
**Increased Variability in Spontaneous Liver Tumor Incidence in Rat Strains**

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Starting Year of trial | Adenomas | Carcinomas | Combined | Adenomas | Carcinomas | Combined |
| | | | Han Wistar strain (CRO 3) | | | |
| 1981–1982 | | | | | | |
| Range | 0–2% | 0% | 0–2% | 0–2% | 0% | 0–2% |
| *n* | 250 | 250 | 250 | 250 | 250 | 250 |
| 1991–1993 | | | | | | |
| Range | 2–12% | 0% | 2–12% | 1–10% | 0–2% | 1–12% |
| *n* | 340 | 340 | 340 | 319 | 319 | 319 |
| | | | BASF Wistar strain | | | |
| 1984–1985 | | | | | | |
| Range | 0–2% | 0–8% | 0–10% | 0–2% | 0% | 0–2% |
| *n* | 320 | 320 | 320 | 320 | 320 | 320 |
| 1993–1998 | | | | | | |
| Range | 0–20% | 4–10% | 5–24% | 4–15% | 0–2% | 5–15% |
| *n* | 350 | 350 | 350 | 350 | 350 | 350 |
| | | | Sprague-Dawley rats (CRO 8) | | | |
| 1985–1986 | | | | | | |
| Range | 0–2% | 0% | 0–2% | 0–2% | 0% | 0–2% |
| *n* | 200 | 200 | 200 | 200 | 200 | 200 |
| 1992–1994 | | | | | | |
| Range | 1–8% | 0–7% | 2–14% | 0–10% | 0–3% | 0–13% |
| *n* | 380 | 380 | 380 | 379 | 379 | 379 |
| | | | Sprague-Dawley rats (CRO 2) | | | |
| 1986–1987 | | | | | | |
| Range | 0–4% | 0–4% | 0–6% | 0–2% | 0% | 0–2% |
| *n* | 400 | 400 | 400 | 400 | 400 | 400 |
| 1993 | | | | | | |
| Range | 2–10% | 0–7% | 2–16% | 2–5% | 0% | 2–5% |
| *n* | 175 | 175 | 175 | 175 | 175 | 175 |

The incidence of spontaneous liver tumors in $B6C3F_1$ mice in BASF studies is similar or somewhat lower those reported by other laboratories for this strain of mouse. B6C3F1 mice were reported (Gopinath, 1994) to have a spontaneous incidence of liver cell adenomas of 10.3% in males and 4.0% in females in the long-term bioassay. The values for carcinomas were 21.3% in males and 4.1% in females. The tumor incidence

**TABLE 5**
**Spontaneous Liver Tumor Incidence Observed in $B6C3F_1$ and C57 BL Mice, at BASF Compared with Those Observed in Basf Wistar Rats in Recent Carcinogenicity Trials**

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Starting Year of trial | Adenomas | Carcinomas | Combined | Adenomas | Carcinomas | Combined |
| | | | $B6C3F_1$ mice (BASF)[a] | | | |
| Mean | 6.5% | 10.3% | 16.8% | 3.4% | 0.8% | 4.2% |
| Range | 0–14% | 4–22% | 14–36% | 0–10% | 0–6% | 0–12% |
| *n* | 600 | 600 | 600 | 650 | 650 | 650 |
| | | | C57BL mice (BASF) | | | |
| Mean | 1.25% | 2.0% | 3.25% | 0.5% | 0.5% | 1% |
| Range | 0–6% | 0–8% | 0–12% | 0–2% | 0–2% | 0–2% |
| *n* | 400 | 400 | 400 | 400 | 400 | 400 |
| | | | BASF Wistar rats | | | |
| 1993–1998 | | | | | | |
| Mean | 9.71% | 5.71% | 15.43% | 7.14% | 0.29% | 7.43% |
| Range | 0–20% | 4–10% | 5–24% | 4–15% | 0–2% | 5–15% |
| *n* | 350 | 350 | 350 | 350 | 350 | 350 |

[a] Recently finalized studies.

in control B6C3F1 from the NTP indicate the following values: males, adenomas, 29.4% (range 4–60%); carcinomas, 17.9% (range 6–29%); females, adenomas, 17.3% (range 2–50%); carcinomas, 8.4% (range 0–20%) (Hasemann *et al*, 1998).

The most recent profile of "spontaneous" liver tumors in BASF Wistar rats is remarkably similar to that of BASF B6C3F$_1$ mice, suggesting that this rat strain has become equally sensitive to the development of liver tumors, and, possibly, to enhancement of liver tumor formation by nongenotoxic substances in carcinogenicity trials. As far as females are concerned, the BASF Wistar rat appears to have become even more susceptible to the development of spontaneous liver tumors than B6C3F$_1$ mice.

*Nongenotoxic Rodent Liver Carcinogens in NTP Trials (Table 6)*

The susceptibility of F344 rats and B6C3F$_1$mice to nongenotoxic liver carcinogens was compared. Positive liver carcinogenicity trials with 22 substances reported to be devoid of genotoxic potential in short-term mutagenicity assays, conducted by the NTP in F344 rats and B6C3F$_1$ mice, were analyzed.

The F344 rat was found to be less susceptible to nongenotoxic liver carcinogens than B6C3F$_1$ mice. Approximately 25% of the compounds tested positive in F344 rats, and approximately 75% tested positive in B6C3F$_1$ mice. Nearly 60%, i.e., 13 of 22 substances (5-chloro-*o*-toluidine, piperonyl sulfoxide, zearalenone, di(2-ethylhexyl)adipate, pentachloroethane, benzofuran, *n*-methylolacrylamide, C.I. direct blue 218, salicylazosulfapyridine, tetrahydrofuran, diethanolamine, pyridine, and coconut oil acid diethanolamine condensate), produced unequivocal evidence of liver carcinogenicity in B6C3F$_1$ mice and no evidence of liver carcinogenicity in F344 rats. Approximately 10%, i.e., 2 of 22 substances (1,1,1,2-tetrachloroethane and 5,5-diphenylhydantoin (phenytoin)), produced unequivocal evidence of liver carcinogenicity in B6C3F$_1$ mice and equivocal evidence of liver carcinogenicity in F344 rats. Approximately 20%, i.e., 4 of 22 substances (di(2-ethylhexyl)phthalate, chlorinated paraffins: C12, 60% chlorine, chlorendic acid, and methyleugenol), produced unequivocal evidence of liver carcinogenicity in B6C3F$_1$ mice and F344 rats.

Approximately 15%, i.e., 3 of 22 substances (11-aminoundecanoic acid, methyl carbamate and monuron),

**TABLE 6**
**Nongenotoxic Rodent Liver Carcinogens in NTP Studies**

| Chemical | Report | Route | F344 Rats | | B6C3F$_1$ Mice | |
|---|---|---|---|---|---|---|
| | | | M | F | M | F |
| 5-Chloro-*O*-Toluidine | 1979 | Feed | − | − | + | + |
| Piperonyl Sulfoxide | 1979 | Feed | − | − | + | − |
| Zearalenone | 1982 | Feed | − | − | − | + |
| 11-Amino undecanoic acid | 1982 | Feed | + | − | − | − |
| Di(2-ethylhexyl)phthalate | 1982 | Feed | − | + | + | + |
| Di(2-ethylhexyl)adipate | 1982 | Feed | − | − | + | + |
| Pentachloroethane | 1983 | Gavage | − | − | + | + |
| 1,1,1,2-Tetrachloroethane | 1983 | Gavage | ± | − | + | + |
| Chlorinated paraffins: C12, 60% chlorine | 1986 | Gavage | + | + | + | + |
| Chlorendic acid | 1987 | Feed | + | + | + | − |
| Methyl carbamate | 1987 | Gavage | + | + | − | − |
| Monuron | 1988 | Feed | + | − | − | − |
| Benzofuran | 1989 | Gavage | − | − | + | + |
| *N*-Methylolacrylamide | 1989 | Gavage | − | − | + | + |
| 5,5-Diphenylhydantoin (phenytoin) | 1993 | Feed | ± | − | − | + |
| C.I. direct blue 218 | 1994 | Feed | − | − | + | + |
| Salicylazosulfapyridine | 1997 | Gavage | − | − | + | + |
| Tetrahydrofuran | 1998 | Inhalation | − | − | − | + |
| Diethanolamine | 1999 | Topical | − | − | + | + |
| Pyridine | 2000 | Water | − | − | + | + |
| Methyleugenol | 2000 | Gavage | + | + | + | + |
| Coconut oil acid diethanolamine cond. | 2001 | Topical | − | − | + | + |
| Number of unequivocally positive studies | | | 6/22 | 5/22 | 16/22 | 17/22 |
| Percentage of unequivocally positive studies | | | 27% | 23% | 73% | 77% |
| Mouse liver carcinogens only | | | 13/22 | | 59% | |
| Mouse liver carcinogens and equivocal rat liver carcinogens | | | 2/22 | | 9% | |
| Mouse and rat liver carcinogens | | | 4/22 | | 18% | |
| Rat liver carcinogens only | | | 3/22 | | 14% | |

*Note.* +, Clear evidence of liver carcinogenicity. ±, Some evidence of liver carcinogenicity. −, No evidence of liver carcinogenicity.

**TABLE 7**
**Nongenotoxic Rodent Liver Carcinogens in BASF Studies**

| Chemical substance | Route | BASF Wistar rat | | BASF mice | | |
|---|---|---|---|---|---|---|
| | | Result | LOEL (ppm) | Strain | Result | LOEL (ppm) |
| 1 | Feed | − | − | C57BL | + | 500 m |
| 2 | Feed | + | 4,500 m | C57BL | + | 8000 f |
| 3 | Feed | + | 8,000 m, f | C57BL | − | − |
| 4 | Feed | + | 10,000 f | C57BL | − | − |
| 5 | Feed | + | 3,000 m<br>4,000 f | C57BL | + | 5000 f |
| 6 | Feed | + | 8,000 m | C57BL | − | |
| 14 | Feed | − | − | $B6C3F_1$ | + | 200 f |
| 21 | Feed | No rat study | | $B6C3F_1$ | + | 7200 f, m |
| Positive in rats | | 5/7 | | | 71% | |
| Positive in C57BL mice | | 3/6 | | | 50% | |
| Positive in $B6C3F_1$ mice | | 2/2 | | | 100% | |
| Positive in C57BL mice only | | 1/6 | | | 17% | |
| Positive in rats only | | 3/6 | | | 50% | |
| Positive in rats and C57BL mice | | 2/6 | | | 33% | |

*Note.* +, Clear evidence of liver tumor formation. −, No evidence of liver tumor formation. f, female. m, male.

produced unequivocal evidence of liver carcinogenicity in F344 rats and no evidence of liver carcinogenicity in $B6C3F_1$ mice.

*Nongenotoxic Rodent Liver Carcinogens in BASF Trials (Table 7)*

In carcinogenicity trials conducted at BASF over the past 15 years, a total of 23 nongenotoxic chemicals were tested in Wistar rats and C57Bl or $B6C3F_1$ mice (Table 7). A total of 8 (35%) chemicals tested positive for liver tumors in either rats or mice. Five chemicals were positive in Wistar rats. Three of these rat liver carcinogens produced no evidence of liver carcinogenicity in C57Bl mice. The other two rat liver carcinogens tested positive for liver tumors in Wistar rats and C57Bl mice. One chemical produced evidence of liver carcinogenicity in C57Bl mice and no evidence of liver carcinogenicity in Wistar rats. Two chemicals produced evidence of liver carcinogenicity in $B6C3F_1$ mice and no evidence of liver carcinogenicity in Wistar rats.

**TABLE 8**
**Liver Tumor Induction in Rats and Mice by Nongenotoxic Liver Carcinogens in BASF and NTP Trials**

| Nongenotoxic liver carcinogen | BASF studies[a] | | NTP studies[b] | |
|---|---|---|---|---|
| | Ratio | % | Ratio | % |
| Carcinogenic in rats | 5/6 | 83% | 6/22 | 27% |
| Carcinogenic in mice | 3/6 | 50% | 17/22 | 77% |
| Mouse liver carcinogen only | 1/6 | 16% | 13/22 | 59% |
| Mouse liver Carcinogen and equivocal rat liver carcinogen | — | — | 2/22 | 9% |
| Mouse and rat liver carcinogen | 2/6 | 33% | 4/22 | 18% |
| Rat liver carcinogen only | 3/6 | 50% | 3/22 | 14% |
| Liver tumour susceptibility | BASF studies | | NTP studies | |
| | Males | Females | Males | Females |
| Spontaneous liver tumour incidence mice | 3.25% | 1.0% | 42.2% | 23.6% |
| Spontaneous liver tumour incidence rats | 15.43% | 7.43% | 2.81% | 0.67% |

[a] Strains used: Wistar rats and C57Bl mice.
[b] Strains used: F344 rats and $B6C3F_1$ mice.

*Susceptibility of Rat and Mouse Strains to Liver Tumor Induction by Nongenotoxic Rodent Liver Carcinogens in BASF and NTP Trials (Table 8)*

The susceptibility of the BASF Wistar rat strain to the induction of liver tumors by nongenotoxic liver carcinogens was found to be very similar to that of the NTP B6C3F1 mouse strain (Table 8). Approximately 80% of the nongenotoxic liver carcinogens produced evidence of liver carcinogenicity in (BASF) Wistar rats and (NTP) $B6C3F_1$ mice, and approximately 50–60% of the nongenotoxic liver carcinogens produced evidence of liver carcinogenicity in (BASF) Wistar rats and (NTP) $B6C3F_1$ mice only. Approximately 15% of the nongenotoxic liver carcinogens produced evidence of liver carcinogenicity in (BASF) C57Bl mice and (NTP) F344 rats only. Approximately 30% of the nongenotoxic liver carcinogens produced evidence of liver carcinogenicity in (BASF) C57Bl mice and Wistar rats and (NTP) F344 rats and $B6C3F_1$ mice.

## CONCLUSIONS

The susceptibility to spontaneous liver tumor formation is strikingly similar in Wistar rats and $B6C3F_1$ mice employed by BASF for carcinogenicity trials.

**TABLE 9**
**Number of Preneoplastic Liver Foci per cm$^2$**

| Source of information | Strain | Age (in months) | No. foci/ cm$^2$liver |
|---|---|---|---|
| BASF | Wistar | 22 | $13.44 \pm 7.29$ |
| Kraupp-Grasl *et al.*, 1990 | Wistar | 16.5 | $1.2 \pm 1.1$ |
| Schulte-Hermann *et al.*, 1983 | Wistar | 17 | 0.77 |
| Schulte-Hermann *et al.*, 1983 | Wistar | 24 | 1.75 |
| Weber and Bannasch, 1994 | Sprague-Dawley | 18.5 | $5.21 \pm 1.07$ |
| Harada *et al.* 1989 | F 344 | 18 | $6.5 \pm 0.6$ |
| Harada *et al.* 1989 | F 344 | 18 | $8.3 \pm 1.3$ |

Especially in the bioassays performed in the 1990s the incidence and the range of spontaneous liver tumor formation are nearly indistinguishable in these two species (Table 5). These findings are quite unusual; it is generally believed that the B6C3F1 mouse is far more susceptible to spontaneous liver tumor formation than any strain of rat.

The NTP data base indicates that a much higher proportion of nongenotoxic liver carcinogens tested positive for liver tumors in B6C3F1 mice than in F344 rats (approximately 80% tested positive in mice and approximately 30% tested positive in rats). The sensitivity of BASF Wistar rats appears to be similarly high as that of $B6C3F_1$ mice used by NTP. Thus, the data suggest that the likelihood of liver tumor induction by nongenotoxic liver carcinogens is related to preexisting susceptibility, i.e., the rate of spontaneous liver tumor formation in the test species, and not so much by the test species used. It is of interest to note, in this context, that the total number of preneoplastic foci per square centimeter liver, as determined by H&E staining and microscopic evaluation, in aged (22-month-old) BASF Wistar rats is much higher than that in other rat strains, determined in the same way (Table 9). The standard deviation on preneoplastic foci in livers of BASF Wistar rats was very high, which could explain the tremendous range of spontaneous liver tumor formation (0–32% in males and 0–20% in females) observed in carcinogenicity trials in these rats. Many nongenotoxic chemicals are known to have the potential to increase liver weight. Such effects are frequently caused by hepatocellular proliferation to meet increased functional demands. This growth stimulus in combination with the presence of a substantial number of preneoplastic foci probably increases the likelihood of liver tumor induction. It is interesting to note that the dose levels required to cause an increased incidence of liver tumors in rats in BASF trials are invariably very high (in all cases $\geq$3000 ppm in the diet). This evidence suggests that nongenotoxic liver carcinogens operate by exerting functional pressure on the liver (associated with large-scale biotransformation of the administered chemical), which demands increased functional activity and liver weight increases. Very frequently, these changes result in increased hepatocellular proliferation, which may exacerbate an ongoing spontaneous carcinogenic process in the liver of these rats.

In the criteria used to conclude that an animal carcinogen is not relevant for humans the EU legislation states that "particular attention should be paid to cases where the only available tumor data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence." With respect to liver tumors it is stated that "if the only available tumor data are liver tumors in certain sensitive strains of mice, without any further evidence, the substance may not be classified in any of the categories." However, the sensitivity to liver tumor induction (by nongenotoxic compounds) would certainly not appear to be a species-specific phenomenon limited to susceptible strains of mouse. BASF Wistar rats appear to be as sensitive as $B6C3F_1$ mice, one of the strains to which the EU criteria are being applied. Thus, the analyses provide relevant arguments which exclude a concern for man for nongenotoxic chemicals that only tested positive for liver tumors in BASF Wistar rats.

The data presented here also indicate an increase in the incidence of spontaneous liver tumors (or at least increasing variability in incidence) in BASF Wistar rats, HanWistar rats, and in Sprague-Dawley rats over time. Tumor drift in rats is not an entirely new phenomenon: Haseman *et al.* (1989) described a virtually linear increase of spontaneous tumors in the hematopoietic system in male Fischer F344 rats (NCI/NTP studies) from approximately 10% in 1971 to nearly 50% in 1981. It is conceivable that liver tumor drift is due to a gradual genetic shift selectively favored by breeding procedures. A strong positive correlation between liver tumor development in mice and body weight has been observed (Haseman, 1997), and breeding procedures that selectively favor fast growth could lead to liver tumor drift. Differences in diagnostic criteria by different pathologists are a less likely explanation in view of standardized working procedures at BASF. Also, the food source (caloric value) was not significantly changed at BASF during the data collection period.

Currently, only BASF Wistar rats show a spontaneous liver tumor incidence that is virtually identical to that of a sensitive strain of mouse, but it would appear that other widely used strains of rats (showing signs of increasing variability in liver tumor incidence) could well become more sensitive in years to come.

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