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Annex 1 to CC/06/19

This annex contains experimental results for the published acute studies for the nine chemicals. Also included at the end of the annex is a tabulated form of the studies.

Urethane

1. Since the first observation in 1943 indicated the carcinogenicity of urethane to mice, extensive carcinogenicity testing has been carried out in a large number of laboratories. The leukaemogenic activity of urethane at a single dose of 1mg/g bw, administered S.C. in 1-, 5-, 40 day old Swiss and AKR mice, were studied by Fiore-Donati et al (1962). Leukaemogenic activity was observed in 1 day old (13/60) and 5 day old (7/39) Swiss mice but not in 40 day old treated mice (2/63). Neonatal treatment (day 1) of AKR mice remarkably shortened the latent period of leukaemogenesis in this high leukaemic strain (14/37 mice developed leukaemia within 19 weeks in treated animals, versus 1/60 within 23 weeks amongst controls). The authors suggest that the leukaemogenic activity exhibited by urethane in these experiments was strictly dependent on age, since no comparable results could be obtained following administration of urethane to adult mice by themselves or others. Chieco-Bianchi et al (1963) considered the role of age in the neoplastic response of the liver cells to urethane in the Swiss mouse strain, a strain with low incidence of spontaneous hepatomas. Groups of mice received a single dose of urethane (1mg/g bw) at the age of 1, 5, 20 and 40 days old by S.C injection. All surviving mice were killed at week 60. By the 60th week, 13/15 (87 %) and 9/13 (70 %) of male Swiss mice treated with urethane at day 1 and day 5 respectively had developed liver tumours. Males treated with urethane at 20 or 40 days developed liver tumours in 8 % and 0 % respectively. In females, no hepatomas occurred in the groups treated at day 20 or 40 but 9 % and 11 % of females developed hepatomas in groups treated at day 1 or day 5 (Chieco-Bianchi et al.,1963).

2. Groups of B6AF1 strain of mice exposed to 1 mg/g bw of urethane, administered as a single dose by stomach tube was investigated by Klein et al., (1966). The animals received the dose on day 1, 7, 14, 21 and 28 following birth and the animals were observed for 16/17 months. The incidence of pulmonary adenomas was 84-100 % in all groups, irrespective of age at treatment compared with 24-36% in controls. No sex difference was observed in the incidence or number of tumours/mouse. The incidence of hepatomas was, however, related to the day of administration of the dose, being highest at 7 days of age. Treatment with urethane on day 1 resulted in the development of hepatomas in both males and females, 45 and 35 % respectively. When treatment was initiated on day 7, there was a high incidence of hepatomas and a difference existed between the sexes, the males appeared more susceptible than females (91 % versus 77 %). Furthermore when treatment was begun on day 14, the sex difference became more pronounced and a similar relationship was noted when urethane was administered on day 21. (80 % v 43 % at day 14; 57 v 5 % at day 21). Both the tumour incidence and average no of tumours per mouse increased approximately 2 fold for each sex when treatment was changed from day 1 to day 7. When treatment was delayed to day 14, males showed a lower incidence as compared to day 7. However, there was an appreciable decline in incidence among the females between day 14 and day 7. Tumour incidence and average number of tumours/mouse

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declined for both sexes at day 21, declined for males at day 28 also but there was no change for females at day 28, as values had essentially reached control levels.

3. Similar results to those of Klein et al (1966) were obtained by Gargus et al (1969) for lung adenomas, where treatment of newborn Swiss mice to a single dose of 1 mg/g or 1.5 mg/g bw subcutaneously, sacrificed at 6 months, induced a near 100 % incidence of lung adenomas in this strain. DeBenedictis et al. (1962) investigated the influence of age following administration of urethane (1 mg/g bw) on lung carcinogenesis in Swiss mice (both male and female). The mice were either treated on day 1, day 45 or as adult mice with a single SC injection and sacrificed on day 20, 45, 90 after treatment. The % animals developing adenomas, treated as newborns, increased with time after treatment. While at 20 days, 20 % of mice, treated at birth, had adenomas, by 90 days all the mice had developed adenomas. In animals aged 45 days at treatment, the incidence was somewhat lower than in mice injected at birth. In male adults, the incidence of lung adenomas was found not to exceed significantly above control animals of the same age.

4. Pietra et al (1961) investigated the effect of subcutaneous and intraperitoneal injections of urethane, at two doses (0.04 mg/g bw and 1mg/g bw) on Swiss mice injected within 24 hours of birth. None of the mice treated with 0.04 mg/g bw of urethane, either SC or IP, showed evidence of development of malignant lymphomas. However, treatment with 1 mg of urethane, either SC or IP, showed evidence of approximately 20 % of the mice developing malignant lymphomas. Treatment of the newborn mice with 0.04 mg of urethane lead to 40 % of mice developing pulmonary adenomas and this increased to 75% (71.4 and 80 %) of mice developing pulmonary adenomas after treatment with 1 mg urethane.

5. Liebelt et al (1964) investigated the effect of a single IP injection of urethane on newborn and young adult mice (C3H/f) of both sexes. Urethane injected at the newborn age at a dose of 0.8 mg/g bw caused an increase in the incidence of hepatomas (90 %) in males. These tumours occurred either alone or in association with other tumours (lung and reticular tissue neoplasm). In newborn females, the incidence of hepatomas was 46 % (18/39) as compared with 1 % of controls. Five animals had only hepatomas, whereas the remaining animals had either reticular tissue neoplasm or lung tumours in addition to liver tumours. One mouse developed ovarian cancer and another developed both stomach and liver tumours following treatment at the newborn age. Males treated at 8-10 weeks of age at a dose of 1 mg/g bw had an incidence of hepatomas of 24 % (6/25) and of lung tumours at 8 %. Increased incidence in liver tumours was not statistically significant. 4/32 of the females receiving urethane at 8-10 weeks developed reticular tissue neoplasms. Although this was a slight increase above controls, it was not statistically significant. No hepatomas or lung tumours were observed in any of the treated females at 8-10 weeks of age.

Ethyl-nitrosourea

6. Single oral administration of ENU at a dose of 10 mg/kg bw in BD-1X rats (10 days old) was investigated by Cravioto et al (1974). Oral treatment with ENU induced neural tumours in 42 % of rats treated (8/19). There were also tumours observed in the spinal cord, cerebellum or peripheral nervous system. Ten day old BD-1X rats were

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given a single oral dose of 10, 20, 40 and 80 mg/kg bw ENU in a study by Druckrey et al (1970). Malignant neural tumours were induced in the brain, spinal cord and peripheral nervous system in 75/80 animals. At the lowest dose of 10 mg/kg bw, 23/26 animals developed neurogenic tumours (21 with brain tumours). At 20 mg/kg bw, 17/18 rats had neurogenic tumours, 19/20 rats at 40 mg/kg bw and all 16 animals treated with 80 mg/kg bw had developed neurogenic tumours.

7. Cravioto et al (1974) also investigated the effect of a single intracerebral injection of 10 mg/kg bw in Long-Evans rats at day 1 following birth. ENU given intracerebrally induced neural tumours in almost all animals treated 10/11 (91 %). Tumours were also seen in the spinal cord, cerebellum and peripheral nervous system. In a study by Druckrey et al.(1970) rats of the BD-1X strain were injected subcutaneously with single doses of 5, 10, 20, 40, 80 mg/kg bw ENU at birth and at 10 days old rats received a single oral dose of 10, 20, 40 and 80 mg/kg bw of ENU. Mainly tumours of the central and peripheral nervous system were produced. At the lowest dose (5 mg/kg bw), 9/28 rats had tumours of the nervous system, at 10 mg/kg bw, 70 % of the animals had tumours of the nervous system. At higher doses, all animals except one had tumours of the nervous system. No difference in response was observed between newborn and 10 day old animals. Treatment of 30 day old BD-1X rats with a single *IV* dose of 20, 40 and 80 mg/kg bw produced tumours predominantly of the central and peripheral nervous system. With 20 mg/kg bw, 18/29 rats had tumours of the nervous system, at 40 mg/kg bw, 15/18 and at 80 mg/kg bw 16/17 rats had developed tumours of the nervous system. More tumours outside of the nervous system including the uterus, liver and kidney were observed (Druckrey et al., 1970).

8. Naito et al (1981a) investigated the effect of a single dose of 40 mg/kg ENU, injected subcutaneously to newborn Wistar rats, within 24 hours of birth. The subcutaneous administration of ENU into newborn rats resulted in 30 % incidence of spinal cord tumours as early as 2 months after treatment. After 6 months, the incidences of spinal cord and spinal root tumours were 64 % and 46 %. The overall incidence of spinal tumours, 6 months after treatment was 86 %. In another study, Naito et al (1981b) investigated the effect of age on the differences and changes in susceptibility in various parts of the nervous system in the perinatal period after a single dose of 40 mg/kg bw ENU, injected subcutaneously. Animals were treated at 24 hours, 1, 2, 3, 4 weeks after birth. The incidence of neurogenic tumours in rats treated within 24 hours after birth was 100 % but this gradually declined from 81 % in groups treated at week 1 to 40 % in rats treated at week 6. The incidence of brain tumours was high in newborns at 68 % and the incidence was 78 % in animals treated at week 1, 72 % in animals treated at week 2 but it was lower in animals treated at week 3 (37 %) and rats treated at week 4 (36 %). This data indicates that the susceptibility of the developing nervous system was not uniform in all parts of the nervous system. The brain showed a high susceptibility during the neonatal period and maintained a high susceptibility up to 4 weeks of age. The susceptibility of the spinal cord was high in neonatal animals and in one week old rats but it fell thereafter.

9. A study by Bosch (1977) compared the effect of a single IP dose of 20 mg/kg bw ENU given to 8 day old and 30 day old WAG strain rats. It was found that in the perinatal period (8 days), there was a high yield of neural tumours (105 tumours in 21

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rats) in comparison to rats treated at day 30 where the ratio was only 14 tumours in 17 animals. The incidence of neural tumours within the perinatal period was 100 % compared with 58 % in rats treated at day 30. Grossi-Paoletti et al. (1972) investigated the effect of a single ENU dose (50 mg/kg bw), either administered intracerebrally or subcutaneously on the induction of tumours of the nervous system in Long Evans rats. The incidence of tumours of the nervous system was higher in all groups ranging from 81-86%. In a small number of animals, non-neurogenic tumours were present. Similar experiments by Grossi-Paoletti et al. in Sprague-Dawley rats, treated with ENU at 50 mg/kg bw, also developed brain tumours but 30 % of the rats died within 2 months of treatment.

10. Jones et al. (1971) studies the incidence, location, structure and histogenesis of rat neural tumours resulting from a single neonatal subcutaneous injection of ENU, at a dose of 10 mg/kg bw in random bred Wistar strain albino rats and hooded Norway rats. Of the 34/38 surviving rats, 33 developed a wide variety of tumours of the CNS or the PNS or both after 197-680 days. A total of 79 neural and extraneural tumours were found in the 33 rats. There were markedly selective for the nervous system, where 71 tumours were located. 87 % developed tumours in the CNS and 13 % in the PNS. There was no difference between the sexes or strain of rats. Non neural tumours were found in 11 % of animals and comprised of three pituitary tumours, a trigeminal nerve myoblastoma, three breast tumours and a leydig cell tumour of the testis.

Benzo(a)pyrene

11. A single intragastric administration of 0.2 mg BaP per mouse (6 mg/kg bw) in PEG produced a total of 14 tumours in 5/11 female albino mice. Tumours appeared following a single dose of 0.05 mg/mouse (1.5 mg/kg bw) and 0.12 mg/mouse (3.6 mg/kg bw) in 0/9 and 2/10 mice (Pierce, 1961). A single oral administration of 100 mg BaP to 50 day old female Sprague-Dawley rats produced mammary tumours in 8/9 rats (Huggins and Yang, 1962).

12. In a dose-response study by Bryan and Shimkin (1943) the effect of a single dose of BaP in a dose range of 0.00195 to 8 mg was given subcutaneously to C3H mice. The mice were 1.5 to 3 months old at the time of injection. The induction of sarcomas was investigated. No tumours were found following doses of 0.031 mg or less whereas 4/20 C3H mice developed tumours of 0.062 mg, with higher doses leading to higher tumour incidences. The average minimal latent period was 3 months. It was noted that in the dose region below 2 mg, the mean latent period increased as the dose decreased. The mean latent period was not correlated with dose in the region of 2 to 8 mg. In a more recent dose response study by Hieger (1959) BaP, dissolved in 1:9 cholesterol:olive oil mixture a dose of 0.00004 mg of BaP was ineffective in producing sarcomas in C57 mice. A dose of 0.0004, 0.004, 0.04 mg BaP produced sarcomas in 1/50, 5/50 and 23/50 mice.

13. Induction of hepatomas and/or lung adenomas and occasional tumours of other sites in mice of different strains were recorded following administration of BaP during the 1st days of life in different solvents. Roe and Walters (1968) injected 20-40 µg of BaP subcutaneously into outbred Swiss mice on the first day of life. The effect of hepatoma induction was confined to male mice. At 20 µg, 2/16 males developed

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hepatomas, while at 40 ug BaP, 6/25 males developed hepatomas. The induction of hepatomas in female mice was the same as for control animals. Toth and Shubik (1967) investigated the effects of BaP, following a subcutaneous injection of 200 µg of BaP into newborn AKR mice. This strain of mice has a high background incidence of malignant lymphomas. The no. of untreated AKR males and females with malignant lymphomas was 88 and 95%, with a latent period of 43.5 and 39.7 weeks, respectively. In BaP injected females, 70 % developed lymphomas with an average latent period of 31.9 weeks. In BaP injected males, 86.5 % developed malignant lymphomas with an average latent period of 37 weeks. In control animals, only 1 developed a lung adenoma. In the BaP injected mice, lung adenomas were observed in 5 females (25%) and in 15 males (40.5 %). Huggins and Yang (1962) reported that a single oral dose of 100 mg benzo[a]pyrene administered by gavage induced mammary tumors in 8/9 female Sprague-Dawley rats. The only studies in which single doses of BaP resulted in a high incidence of malignant lymphoma in adult mice were those of Rask-Nielsen (1948, 1950) administered carcinogens either in small doses (0.02 mg.) directly into the target organ (thymus or lung) or in large doses (0.5 mg.) subcutaneously.

14. Among the many positive study, with SC injections of BaP to rats, only the study of Oberling et al, (1939) was a dose response study. Single injections of 0.05, 0.1, 0.5 and 1.0 mg BaP in olive oil produced tumours respectively in 1/7, 4/31, 9/17 and 64/69 in rats.

7,12, dimethylbenzanthracene

15. A preliminary study in 1959 by Pietra et al showed that a small single dose of DMBA (30-40 µg) injected into newborn mice lead to a high incidence of malignant lymphomas. In a follow-up study in 1961, Pietra et al investigated the effect of a SC injection and an IP injection on the induction of malignant lymphomas and pulmonary adenomas, administered at a dose of 30-40 µg within 24 hours of birth. There was a high incidence of malignant lymphomas in animals receiving DMBA by SC and IP. The mice treated with DMBA showed a somewhat lower incidence of malignant lymphomas. There was a high incidence of pulmonary tumours in mice treated with DMBA at birth. In a second experiment, 8 week old mice were injected with 0.9 mg of DMBA subcutaneously. Most animals were observed until they died spontaneously. A much lower incidence of malignant tumours and pulmonary adenomas were observed in animals receiving DMBA at 8 weeks rather than as newborn mice.

16. Roe et al, (1961) found that injection of a single dose of DMBA subcutaneously into newborn CBA mice lead to a high incidence of parenchymal cell hepatomas. Roe and Walters (1968) also investigated the effect of DMBA on 101 strain mice at a dose of 15 ug DMBA and CBA mice at a dose of 45 µg DMBA. In both strains of mice, only the male animals were affected. In CBA mice, parenchymal cell hepatomas were seen as early as 28 weeks of life and in 16/27 mice but in the 101 strain, no hepatomas were seen before the 50th week and they were observed in 9/79 mice.

Diethylnitrosoamine

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17. A single iv injection of 280 mg/kg bw DEN to BD-1X rats produced kidney tumours in all 4 animals treated (2 had kidney adenomas and 2 had kidney carcinomas) and one carcinoma in the ovary was also observed (Druckrey et al., 1964). Treatment of the BD-1X rats with a single oral dose of 280 mg/kg NDEA lead to the development of liver carcinomas in 1/3 animals treated (Druckrey et al., 1964). A dose response study was performed by Mohr and Hilfrich (1972) in male and female Sprague-Dawley rats. Doses of 1.25, 2.5, 5.0, 10, 20, 40, 80, 160 mg/kg bw were given as a single iv dose. One female given 1.25 mg/kg bw developed an adenoma of the kidney. Dose levels of 40 mg/kg bw lead to 3/10, and above doses increased the tumour incidence and decreased survival time. At 80 mg/kg bw, 6/10 females had developed kidney tumours while 8/10 had at 160 mg/kg bw DEN. Males were less sensitive to DEN than females, with 3/10 males developing kidney tumours at 40, 80, 160 mg/kg bw NDEA.

Dimethylnitrosoamine

18. In a study by Cardesa et al (1974a, 1974b), a dose response was observed when 8 week old Swiss mice were administered a single subcutaneous dose of DMN at 0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw. The following results were observed: 33/218 (15 %) of control animals developed lung tumours (adenomas and carcinomas), 7/40 (17 %) of animals at 0.5 mg/kg bw, 11/38 (29 %) at 1.0 mg/kg bw, 12/34 (35 %) at 2.0 mg/kg bw, 15/37 (39 %) at 4.0 mg/kg bw and 26/39 (67 %) of animals developed lung tumours at 8.0 mg/kg bw. A single dose of DMN, given at 5, 10, 15 mg/kg bw, given subcutaneously to RF mice, induced lung tumours (adenomas and papillary carcinomas) in 9/18, 16/19 and 4/5 animals respectively, compared with 25/52 in controls (Clapp et al., 1968).

19. In a study by Magee and Barnes (1959), administration of a single dose of DMN (30 mg/kg bw) (LD 50 dose) to rats lead to the development of kidney tumours in 3/15 rats but no liver lesions nor liver tumours were observed. Terracini and Magee (1964) investigated the effect of a single administration of DMN to newborn and 1 week old Wistar Porton rats at a dose of 125 µg of NDMA or 250 µg of NDMA. The dose was administered by subcutaneous injection. All rats receiving the higher dose of 250 µg died before the end of the second week. Out of the 44 animals given 125 µg of DMN at less than hour hours, 29 were alive at weaning. Of these, 19 were still alive and under observation at 53 weeks. In the only litter injected with 125 ug of DMN at 1 week of age 4 or the 8 were still under observation. Among the 14 animals that died after weaning, and at the time of publication (10 from the <24 hour treatment and 4 from the 1 week treatment), 11 bore tumours. Renal tumours were found in nine animals, one of which had both kidneys involved and another also had a hepatoma. Another animal died with a hepatoma and the remaining tumour bearing rate had an abdominal malignant lymphoma.

Methyl-nitrosourea

20. In a study by Leaver et al (1969) a single oral dose of MNU, at a dose level of 90 mg/kg bw, was given to both male and female Wistar rats of the Porton strain to investigate the induction of kidney tumours in the treated animals. The animals

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received the dose by intragastric tube and were killed at 6, 10, 14, 20 or 29 weeks or kept until they died spontaneously. Animals that were obviously ill were killed immediately. 9/40 rats died within a month of the single dose and the remainder died or were killed between 18 and 61 weeks after treatment. All but 4 of the animals developed tumours, which occurred primarily in the kidney (15/40), squamous stomach (24/40), small intestine (6/40), large intestine (16/40) and skin (8/40). 12 rats had tumours at more than one site. Leaver et al (1969) did not find any apparent differences in the distribution of the different types of tumours between the sexes.

21. Terracini and Testa (1970) undertook a study to compare the susceptibility of newborn mice (C57BL and C3Hf1) and rats (wistar) with more mature animals (5 weeks old) following treatment with a single dose of MNU. Animals were treated within 24 hours after birth or at 35 days with a dose of 50 µg/g bw of MNU intraperitoneally. In terms of survival, 11/45 mice treated at birth died before weaning compared with 3/34 controls. Early deaths did not occur in mice or rats treated at 5 weeks of age. A high carcinogenic activity of MNU with the production of a broad spectrum of tumours was observed. Only 7% of mice treated at birth and 21% of mice treated at 5 weeks were tumour free compared with 97% of control animals. In rats, treated either as newborns or at 5 weeks, only 3/52 animals were tumour free at death. Lymphosarcomas were the most common tumours found in mice. Among the mice treated at birth, the incidence of lymphosarcomas in both sexes ranged from 50-60 % with an average age at death of 17-18 weeks. The incidence of lymphosarcomas in mice treated at 5 weeks of age was 46 % in females and 31 % in males, with an average age of death of 29 weeks in both sexes. Kidney tumours occurred at the same frequency among newborns and 5 week old mice. Lung tumours occurred only in experimental mice surviving the period of high mortality due to lymphosarcomas. Animals treated at birth (16/19 females, 12/15 males) were more susceptible than those treated at 5 weeks (10/35 females, 10/26 males). Hepatomas were only seen in mice and there was a higher susceptibility of males than females to this type of tumour (at birth 1/17 females and 10/12 males). A sharp loss in susceptibility to hepatomas was observed in animals treated at 5 weeks of age, with no hepatomas observed. Forestomach tumour incidence was higher in mice treated at 5 weeks (12/18 in females and 8/22 in males) than those treated at birth (4/17 females and none in males). In comparison, lymphosarcomas were only found in 1/10 rats (1 male) treated at birth and 3/19 (2 male and 1 female) rats treated at 5 weeks of age. Renal tumours were the most common observed neoplasm amongst rats treated either at birth or at 5 weeks. They were bilateral in 11 rats treated at birth and in 1 rat treated at 5 weeks. The incidence of renal tumours among survivors at 20 weeks of age was 74 % in rats treated at birth and 37 % in those treated at 5 weeks.

22. In a dose response study, a lifetime analysis of the carcinogenicity of a single IP dose administration of MNU at 5, 25 and 50 µg/g to newborn C3Hf1 mice, 21 day old mice or 70 day old mice was investigated by Terracini et al (1976). In this paper results were not shown for 5 µg/g of MNU as there was no obvious increase over control levels in the probability of dying from a tumour at any site. No thymic tumours were found in controls or animals given 5 µg/g at day 70. The incidence in mice receiving 50 µg/g MNU at 70 days of age was 21.2 %. Reducing the dose given at day 70 to 25 µg/g MNU produced a decrease in the incidence to 2.5 %. In comparison to treatment at birth and day 21, treatment at day 70 caused a reduction in

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the risk of dying from a thymic tumour. In control animals, 5/59 animals (5 females) died of forestomach tumours. In animals receiving 25 µg/g MNU at day 70, 10/40 died of forestomach tumours (2 with carcinomas and 8 with one or more papillomas of the forestomach). Among the mice receiving 5 µg/g at day 70, the incidence of papillomas of the forestomach was, if anything, lower than controls. It was observed that 10/59 controls died of lung adenomas with a significant proportion 12/20 males dying after treatment with 25 µg/g at day 70. Similarly a large proportion of males 18/30 and 24/41 of females treated with 50 µg/g of MNU at day 70 died of lung adenomas. Renal tumours were observed in 21/71 of mice receiving 50 µg/g of MNU at day 70. Mice exposed at day 70 appeared to be more susceptible than those treated at earlier stages, but the difference was of borderline significance.

23. Kelly et al (1968) investigated the effect of a dose response of MNU on the possibility of producing tumours in the brains of newborn Sprague Dawley rats, general purpose mice and CDF1 mice by direct intracerebral injections of MNU and subcutaneous tissue tumours by a single SC injection. The animals were dosed at 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/animal MNU. In the mice, a single intracerebral or subcutaneous injection of MNU to newborn general purpose and CDF1 mice induced leukaemia and pulmonary tumours. The dose varied from 0.05 to 0.8 mg/mouse but few animals survived for 8 weeks or longer at doses higher than 0.1 mg. Leukaemia was induced in 37-80 % of mice given 0.05 or 0.1 mg of MNU intracerebrally or subcutaneously. Multiple pulmonary tumours were obtained by both route of administration. The incidence varied from 67-100 % and the mean nodule count from 4-9. There was a direct correlation between the dose and response. Although the CDF1 mice were somewhat less sensitive than general purpose mice to induction of leukaemia by MNU, 8-80 % of this strain developed leukaemia after intracerebral or SC injection of MNU. The CDF1 mice were more susceptible than the general purpose mice to pulmonary adenomas induced by MNU. All CDF1 mice at the doses treated had pulmonary adenomas at autopsy, with a mean nodule count of 6-18 nodules/mouse. Given an intracerebral injection of MNU to newborn rats did not lead to the induction of brain tumours or disturbances of the CNS system. One female developed a mammary carcinoma at 14 weeks at 0.4 mg dose and 3 females developed fibrosarcomas, one of which metastasised to the ovary.

Dibenz (a,h) anthracene

24. In an acute study by Bryan and Shimkin (1943), a dose response relationship was observed for sarcomas following treatment of C3H mice with DBA. DBA, dissolved in tricapylin, was given to a group of mice as a single injection. At doses of 0.00019, 0.0078, 0.016, 0.03, 0.06, 0.12, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg the respective incidences of local sarcomas was 2/79, 6/40, 6/19, 16/21, 20/20, 21/23, 19/21, 20/21, 22/22, 19/19, 17/20 and 16/21. Thus the lowest effective dose was 0.0019 mg. When all the mice were considered together, receiving between 0.00019-0.031 mg, DBA induced sarcomas in 30/159, with an average latent period of 3.7 months.

25. A dose-response relationship was established among a group of general purpose/NIH newborn mice receiving SC injections of eight log spaced dose levels ranging from 0.003 to 6.7 µg/mouse (O'Gara et al., 1965). Each mouse received a single SC injection of carcinogen within 12 hours of birth. During the first year of the

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study, mice were not killed unless they developed progressively growing tumours. At 0.08 μg and doses above this, a consistent, dose related appearance of sarcomas were observed. Sarcomas at the injection site were seen sporadically following administration of 0.003 μg - 0.01 μg DBA and were consistently seen at 0.08 μg and above. The incidence of sarcomas was higher in males than in females, though the sarcomas often appeared earlier in females. The incidence of lung adenomas increased at 0.2 μg DBA and above.

26. In a study by Kelly and O’Gara (1961), the carcinogenic response in non inbred albino mice and inbred C3H amdC57BL mice was investigated in newborn, following a single low dose injection given subcutaneously (0.06 mg). In the non inbred albino mice, at 43 % of the mice developed pulmonary adenomas by 8 weeks after injection, 82 % by 16 weeks and 96 % by 24 weeks after injection. The number of tumours/mouse also increased with time from a mean nodule count of 3-6 at 8 weeks to a count of >20 by 24 weeks after injection. This study also contained a dose response study, as measured by the induction of pulmonary tumours following administration of a single SC injection of DBA to newborn mice. The newborn mice received 5 log spaced doses levels of each carcinogen (0.0022, 0.0067, 0.02, 0.06, 0.18 mg) and were sacrificed at 8, 16 and 24 weeks after the injection. The % of mice developing pulmonary tumours and the number of nodules per mouse increased with dose and time after the injection. The lowest dose of 0.0022 mg DBA induced pulmonary tumours in 38% of mice by 8 weeks, with a mean nodule count of 0.8. At this dose, neither the incidence nor the mean nodule count increased with time over the 24 weeks observation period. Maximum response was obtained at dose levels of 0.02, 0.06, 0.18 mg DBA. Mean nodule count was higher at 0.18 mg, though the incidence of tumours was about the same for all three doses. By 24 weeks, virtually all the animals at 0.02 mg or more had pulmonary tumours, with a mean nodule count ranging from 8 at the lowest dose to 40 at the highest dose.

27. O’Gara and Kelly (1965) also examined the effect of age on the susceptibility of the mice to develop pulmonary adenomas. In this part of the study, a single SC injection of 0.06mg of DBA was given to either newborns, sucklings (1 and 3 weeks old) or as young adults (6 weeks of age). The mice were killed at 8, 16 and 24 weeks after injection and examined for pulmonary tumours. Within 8 weeks after injection, pulmonary adenomas had developed in 40 % of mice given DBA as newborns and in only 10% of those given DBA at 1, 3 and 6 weeks of age. The mean nodule count of the different age groups at this time was 3.1 for the newborns and 0.1 for the 1, 3 and 6 weeks old groups. By 16 weeks after the injection, however, mice given DBA at week 1 of age had a somewhat higher incidence (91 vs. 78 %) and a mean nodule count (13.2 vs. 10.3) than animals given DBA as newborns. In contrast, the mice treated at 3 and 6 weeks of age showed little or no increase in either the incidence or mean tumour count throughout the observation period. When the % incidence of pulmonary tumours and the mean nodule count of mice administered DBA are plotted against chronological age of the mice when they are killed rather than after injection of DBA, similarities in the pattern and intensity of response of the newborn and 1 week old groups are comparable to each other and differ considerably from that of the older age groups (3 and 6 weeks old). The newborns and 1 week old mice show a progressive increase in response to carcinogen during the subsequent 24 week observation period, particularly in no of nodules per mouse. The latent period was

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longer however, in 1 week old animals than in newborn (16 weeks vs. 8 weeks). These results are in contrast to those obtained with animals given the carcinogen at 3 and 6 weeks of age, in which the response curves remain low and essentially flat throughout the observation period.

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Table 1. Acute studies

Chemical	Species	Sex	Strain	Age of animal at dosing	Route	Target	Dose Response Data Y/N
3-methylcholanthrene	TD50 values from chronic carcinogenicity data available for rat						
Urethane	TD50 values from chronic carcinogenicity data available for rat, hamster and mouse						
Fiore-Donati et al (1962)	Mice	Both	Swiss	Day 1, 5,40	SC	Leukaemia	No, 1mg/g bw
Fiore-Donati et al (1962)	Mice	Both	AKR	Day 1	SC	Leukaemia	No, 1mg/g bw
Liebelt et al (1964)	Mice	Both	C3H/f	Day 1 and Week 8	SC	Hepatomas, Reticular Tissue Neoplasm, Lung, heamangioma, Ovarian and Stomach	No, 0.8 mg/g bw at birth, 1.0 mg/g bw at 8-10 weeks
Chieco-Bianchi et al (1963)	Mice	Both	Swiss	Day 1, 5, 20 and 40	SC	Hepatomas	No, 1 mg/g bw
Klein et al (1966)	Mice	Both	B6AF1/J	Day 1, 7, 14, 21 and 28	Stomach tube	Hepatomas	No, 1mg/g bw
Pietra et al (1961)	Mice	Both	Swiss	Day 1	SC and IP	Malignant lymphomas and pulmonary adenomas	No, 0.4 mg/g and 1.0 mg/g bw
Gargus et al (1969)	Mice		Swiss	Newborn	SC	Lung adenomas	No, 1mg/g bw, 1.5 mg/g bw
DeBenedictis et al (1962)	Mice	Both	Swiss	Day 1, 45 and adult	SC	Lung adenomas	No, 1mg/g bw

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Ethyl-nitrosourea	TD50 values from chronic carcinogenicity data available for rat						
Cravioto et al (1974)	Rat	Both	Long evans	New born	intra-cerebral	Neural Tumors, spinal cord, cerebellum and peripheral nerves	No 10 mg/kg bw
Cravioto et al (1974)	Rat	Both	BD-1X	10 day old	Oral	Neural Tumors, spinal cord, cerebellum and peripheral nerves	No 10 mg/kg bw
Druckery et al (1970)	Rat		BD-IX	Day 1, 10, 30	Oral, SC and IV	Nervous system (brain, spinal cord and PNS)	Yes 5,10, 20,40,80 mg/kg
Naito et al (1981a)	Rat	Both	Wistar	New born	SC	Spinal Cord tumors, brain tumors and trigeminal tumors	No, 40 mg/kg bw
Naito et al (1981b)	Rat	Both	Wistar	Day 1, 7, 14, 21, 28	SC	Neurogenic tumors	No, 40mg/kg bw
Grossi-Paoletti et al (1972)	Rat	Both	Long-Evans	Day 1	SC IC	Nervous system	No, 50 mg/kg bw
Bosch (1977)	Rat	Both	WAG	Day 8 and Day 30	IP	Nervous system	No, 20mg/kg bw
Jones et al (1971)	Rat	Both	Wistar Norway hooded	Day 1	SC	Nervous system	No, 10 mg/kg bw
Grossi-Paoletti et al., (1972)	Rat	Both	Sprague-dawley	Day 1		Brain tumours	No,50 mg/kg bw

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n-Nitroso-methylurea	TD50 values from chronic carcinogenicity data available for rat and mouse						
Leaver et al (1969)	Rat	Both	Wistar		Oral	Kidney, squamous stomach, SI, large intestine and skin	No, 90 mg/kg bw
Terracini and Testa (1970)	Rat	Both	Wistar	Day 1 and Day 35	IP	Lymphosarcomas, lung adenomas, hepatomas, renal adenomas and forestomach tumours	No, 50 ug/g bw
	Mouse	Both	C57BL and C3Hf1				
Terracini et al (1976)	Mouse	Both	C3Hf1	Day 1, 21 and 70	IP	Thymic tumors, extra-thymic, liver cell tumours, lung adenomas, renal tumours and stomach tumours	Yes 5,25,50 ug/g bw (no data presented for 5)
Kelly et al., (1968)	Rat		Sprague-dawley	Newborn	IC, SC	Brain, pulmonary, leukaemia, mammary	Yes (0,0.05,0.1,0.2, 0.4, 0.8, 1.6 mg/animal)
	Mice		General purpose	Newborn			
	Mice		CDf1	Newborn			
n-Nitrosodimethylamine	TD50 values from chronic carcinogenicity data available for rat and mouse						
Cardesa et al., 1974	Mice	Both	Swiss	8 week old	SC	Lung tumours	Yes, 0.5, 1.0, 2.0, 4.0, 8.0

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							mg/kg bw
Clapp et al, 1968	Mice	Both	RF		SC	Lung tumours	Yes, 0, 5,10, 15 mg/kg bw
Terracini et al., 1964	Rat	Both	Porton	Newborn and 1 week old	SC	Kidney,heaptoma, malignant lymphomas	No, 125 and 250 ug/animal
Magee and Barnes (1959)	Rat	Both	Porton	Not stated in paper, assume early	Oral	Kidney	N
n-Nitrosodiethylamine	TD50 values from chronic carcinogenicity data available for rat						
Mohr and Hilfrich, 1972	Rat	Both	Sprague-dawley	Not stated	iv	Kidney	Yes, 0,1.25, 2.5, 5.0, 10, 20, 40, 80, 160 mg/kg bw
Druckrey et al., (1964)	Rats		BD-1X	Newborn	iv	Kidney	No, 280mg/kg
Benzo(a)pyrene	TD50 values from chronic carcinogenicity data available for rat and mouse						
Vesselinovitch et al (1975)	Mice	Both	BC63F1 C3A/JF1	Days 1, 15 and 42	IP	Lymphoreticular tumors	N
Toth and Shubik (1967)	Mice	Both	AKR	Day 1	SC	Malignant lymphomas	No, 200 ug/mouse
Roe & Walters (1967)	Mice	Both	Swiss albino	Day 1	SC	Hepatomas	Yes (20 and 40 ug)
Rask-Nielsen et al (1948, 1950)	Mice	Both	Street	4 – 7 weeks old	Direct Inj sc	Thymic, Pulmonary, mammary, leukaemia, spindle cell sarcomas,	No, 0.02 mg directly, 0.5 mg sc

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Pierce (1961)	Mice	Female	Albino		Intra-gastric tube		Yes, 0, 0.012, 0.05, 0.2 ug/g bw
Huggins and Yang (1962)	Rat	Female	Sprague Dawley	50 day old	Oral	Mammary	No, 100mg/rat
Byran and Skimkin (1943)	Mice		C3H	1.5 to 3 months old	SC	Sarcomas	Yes, 0.00195 to 8 mg
Oberling et al (1939)	Rats				SC	Tumours	Yes, 0.05, 0.1, 0.5, 1.0 ug
Hieger et al (1959)	Mice					Sarcomas	Yes, 0.00004, 0.0004, 0.004 and 0.04 ug
Dibenz(a,h)anthracene							
TD50 values from chronic carcinogenicity data available for mouse							
Kelly and O’Gara (1961)	Mice	Both	Albino, C3H/P C57BL/6 JN	Newborn, day 7, 21 and 42	SC	Lung, subcutaneous fibrosarcomas, sebaceous-gland adenomas, leukemias and hepatomas	Yes for newborn Albino (0.0022, 0.0067, 0.02, 0.06, 0.18 ug) No, 0.06 mg to C3H/P C57BL/6JN
O’Gara et al (1965)	Mice	Both	Albino	Newborn (within 12 hours of birth)	SC	Pulmonary tumors and Fibrosarcomas	Yes (0.003, 0.01, 0.03, 0.8, 0.2, 0.7, 2.2, 6.7

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							ug/animal)
Pietra et al (1961)	Mice	Both	Swiss albino	Day 1	IP SC	Malignant lymphomas, Pulmonary adenomas	No,30-40 ug/mouse
Byran and Shimkin (1943)	Mice		C3H	1.5 to 3 months old	SC	Sarcomas	Yes, 0.00019 to 8.0 mg
O’Gara and Kelly (1965)	Mice			Newborn, sucklings (1 to 3 weeks old), or young adults (6 weeks old)	SC	Pulmonary tumours	No, 0.06 mg
7,12,dimethylbenz(a)anthracene			TD50 values from chronic carcinogenicity data available for mouse				
Pietra et al (1961)	Mice	Both	Swiss	Day 1 and Week 8	SC and IP	Malignant lymphomas and pulmonary adenomas	No (30-40 ug/ mouse)
Pietra et al (1959)	Mice	Both	Swiss	Day 1	SC and IP	Malignant lymphomas	No (30-40 ug/mouse)
Roe et al (1961)	Mice		CBA	Newborn	SC	Parenchymal cell hepatomas	No,
Roe and Walters (1968)	Mice		CBA	Newborn	SC	Parenchymal cell hepatomas	No, 45 ug/mouse
Roe and Walters (1968)	Mice		101 strain	Newborn	SC	Hepatomas	No, 15 ug/mouse