6 Mathematical Models of Carcinogenesis

6.1 Introduction

6.1.1 Models of carcinogenesis

As discussed in chapters 3 and 4, the process of carcinogenesis is very complex. It is too complex to be described in full detail by a mathematical model. Therefore, modelling carcinogenesis implies simplifications that try to identify the most important features of the process. Resulting predictions can be tested in laboratory experiments with carcinogens or by analyses of epidemiological cohorts. Compared to conventional risk models used in epidemiology, as, e. g, the excess relative risk model, mathematical models of carcinogenesis have the advantage of a straightforward description of complex exposure scenarios. After identification of the action of a carcinogen on the parameters of the model, no additional parameters are necessary to calculate the effects of exposures that may change many times over lifetime or differ for the various members of the study cohort.

Normal cells that have the potential to develop into a cancer cell are called here susceptible. Most stem cells are assumed to be susceptible for a stimulation of the carcinogenic process. Carcinogenesis is generally modelled by a process in which a susceptible normal cell undergoes several alterations to become a cancer cell. In most cases, such alterations are genetic or epigenetic changes. The number of necessary genetic alterations appears to vary from cancer to cancer. In the case of retinoblastoma, two mutations that inactivate both copies of the RB tumour-suppressor gene apparently suffice for tumour development (Knudson 1971, Bishop 1991; Weinberg 1991). In the case of colorectal cancer alterations in three tumour-suppressor genes (*APC, p53* and *DCC*) and one proto-oncogene (K-*ras*) have been observed during progressive stages of tumour development (Fearon and Vogelstein 1990). The presence of four or more mutations in the genesis of colorectal cancer suggests the existence of events that increase the rates of subsequent mutations, i. e. the induction of a genomic instability (Aaltonen et al. 1993; Peltomäki et al. 1993) or mechanisms that inactivate proteins of tumour-suppressor genes, e. g. a dominant negative action, as it has been observed for a mutant form of the p53 protein (Gannon 1990). Also, the increase of the division rate of intermediate cells in the carcinogenic process may lead to an increase of critical mutational events.

Phenotypically altered cells, that are believed to represent intermediate stages of carcinogenesis, may form clones as papillomas of the mouse skin, enzyme altered foci in the rodent liver, or adenomatous polyps in the human colon. Correspond-

ingly, a large family of mathematical models of carcinogenesis contains a clonal expansion of the intermediate cells.

Early models of carcinogenesis (Nordling 1953; Armitage and Doll 1954) were proposed to describe age-specific cancer mortality data that for many cancer types could be approximated by an increase with a power of age. section 6.2 describes these early models, in which carcinogenesis is modelled by the occurrence of a number of cellular transformations. If all transformation rates are small, then a model with k cellular transformations leads to a proportionality of the mortality rate to age to the power of $(k - 1)$.

Section 6.3 describes a further development in modelling of carcinogenesis, namely the explicit implementation of cell proliferation kinetics, as it was introduced by Armitage and Doll (1957). The two-step version of the model was successfully applied to the genesis of retinoblastoma (Knudson 1971). The model was further developed by Moolgavkar and Venzon (1979) by taking account of cell death and differentiation. Two-step models of carcinogenesis with a clonal expansion of intermediate cells are among the simplest models that can describe age and exposure dependencies in many observational data. This is possibly because the complex transition from a normal cell to a malignant cell can be approximated at least in some cases by two rate-limiting events. Independent of this, there is an increasing effort to develop and apply multistep models that take into account cell proliferation kinetics (Moolgavkar and Luebeck 1992; Little 1995; Little et al. 2002; Luebeck and Moolgavkar 2002).

Models of carcinogenesis and effects of environmental agents have been reviewed by Kopp-Schneider (1997) and by Moolgavkar et al. (1999), and the present chapter focuses on basic concepts and on more recent work. The application of mathematical models of carcinogenesis to leukaemia has been explored only to a limited degree and is therefore not treated here. The interested reader may find an analysis of the lymphocytic leukaemia incidence in England and Wales with mathematical models of carcinogenesis in Little et al. (1996).

6.1.2 Hazard and excess risk

In epidemiology, new cancer cases or cancer deaths observed during a given time period among a collective of people are expressed by the cancer incidence rate or cancer mortality rate, respectively. These rates are defined by the number of cases per person-years of observation. In modelling, both rates are often called the hazard $H(a)$, where a denotes the age. The hazard is related to the probability $p(a - t)$ that a susceptible cell has become a malignant cell at age $(a - t)$, where t is the lag time that is necessary for the malignant cell to grow in a detectable tumour (incidence data) or to lead to death (mortality data). If there are n_s susceptible cells in an organ, then the probability $\Psi(a - t)$ that none of these cells has become malignant is

$$\Psi(a - t) = [1 - p(a - t)]^{n_s}. \qquad \text{(eq. 6.1)}$$

In the modelling literature, sometimes the function $\Psi(a)$ is called the survival function $S(a)$. We do not follow this terminology, because the term survival function

$S(a)$ is used here as in the epidemiological literature for all causes of death and not just for cancer (see below).

The hazard of a tumour at age a is defined by the quotient of the number of new cancer cases and the number of people without cancer observed in a time interval, which can be expressed by the probability - that at least one cell has become malignant in the time interval , where the dot denotes the derivative with respect to age a, divided by the probability that up to the time interval no cell has become malignant:

$$H(a) = \dot{\Psi}(a - t) / \Psi(a - t). \qquad \text{(eq. 6.2)}$$

With eq. (6.1) one obtains

$$H(a) = n_s \dot{p}(a - t) / [1 - p(a - t)]. \qquad \text{(eq. 6.3)}$$

Under the influence of a carcinogenic agent, the hazard is conventionally divided into a baseline hazard $H_0(a)$ and the hazard that is attributed to the agent. Fig. 6.1 shows as an example the baseline hazard $H_0(a)$ to get a solid tumour at age a, as it was derived by an adaptation of a model of carcinogenesis to the solid cancer incidence data for the male atomic bomb survivors.

The difference between the total hazard and the spontaneous hazard is called the excess absolute risk (see also chapter 5):

$$EAR(a) = H(a) - H_0(a). \qquad \text{(eq. 6. 4)}$$

The ratio of the excess absolute risk and the spontaneous hazard is called the excess relative risk

$$ERR(a) = H(a)/H_0(a) - 1. \qquad \text{(eq. 6. 5)}$$

In order to calculate cancer risks over given periods of time or lifetime risks, a survival function $S(a)$ is used, which is defined by the probability that a newborn

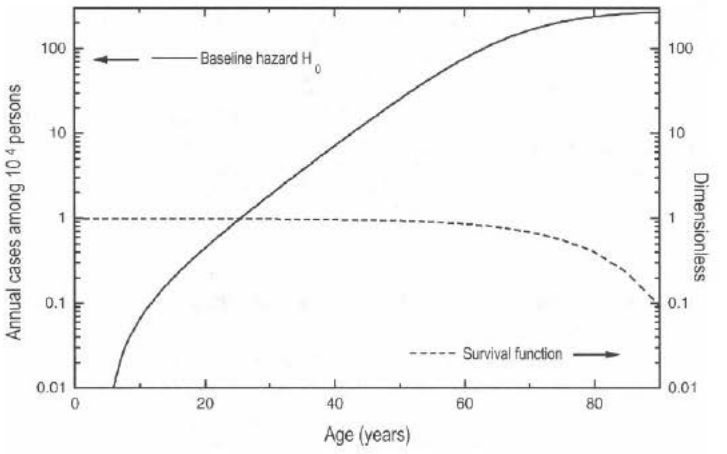

Fig. 6.1 Baseline hazard $H_0(a)$ to obtain a solid tumour at age a among male atomic bomb survivors with negligible radiation exposures, as derived with the TSCE model (see section 6.3.1), and the survival function $S(a)$ for German males in the period 1996–98 (Statistisches Bundesamt 2000).

person will survive until age a. In general, complete and reliable data on survival functions are not available. Therefore the survival function is approximated by products of age dependent death rates for a given calendar year (period) as they are easily available. Fig. 6.1 gives as an example a survival function that has been obtained from German death rates in the period 1996–98, according to which a newborn person has a chance of 9 % to survive until the age of 90.

The baseline lifetime hazard R_0 to get cancer or to die of cancer is defined by

$$R_0 = \int S(a)\, H_0(a)\, \mathrm{d}a. \qquad \text{(eq. 6.6)}$$

For the example in Fig. 6.1 the baseline lifetime hazard R_0 (up to the age 90) has a value of 0.33, i. posure of a carcinogenic agent, the total lifetime hazard R is

$$R = \int S(a)\, H(a)\, \mathrm{d}a. \qquad \text{(eq. 6.7)}$$

The excess relative lifetime risk ERR time due to the exposure is defined by

$$ERR = R/R_0 - 1. \qquad \text{(eq. 6.8)}$$

Note that this is a slightly simplified concept, continuative references are Vaeth and Pierce (1990) and Kellerer et al. (2001).

Hazard functions and related excess relative risks have been derived by applying models of carcinogenesis to epidemiological cohorts and animal experimental data sets with exposures to ionising radiation (section 6.4), chemical carcinogens (section 6.5) and combined exposures (section 6.6). In addition, the sections give some more theoretical derivations as considerations of threshold values or properties of the hazard function for combined exposures with low doses.

6.2 Models without clonal expansion

For most cancer sites, carcinogenesis is considered as a complex process with several cellular transitions. In most cases these cellular transitions are mutations. However, they can be also epigenetic or other cellular events. For some of these transitions, it will not be of importance, which happens first. For other transitions, the sequence might be of importance. In the modelling, mainly two extreme cases, total independence on the sequence of the transitions, and definite sequence of all transitions (Armitage-Doll model) have been analysed.

6.2.1 A model in which the sequence of cellular transitions is inconsequential

Nordling (1953) proposed a model of carcinogenesis in which a cell has to undergo k transitions until it becomes a cancer cell. The order of occurrence of the transitions was not considered to be important. Although Nordling suggested that due to symmetric cell divisions the number of cells with transitions might increase leading to an increasing probability that a further mutation occurs in one of the cells, this effect was not taken into account in the mathematical formulation of the model.

In the following λ_κ denotes the rate of the κ^{th} cellular transition, where κ can have the values 1,..., k. Then the number of cells without the κ^{th} transition will change according to

$$\dot{n}(a) = -\lambda_\kappa(a)\, n(a). \qquad \text{(eq. 6.9)}$$

If the rate λ_κ is constant over lifetime, then the number of cells without the κ^{th} transition is:

$$n(a) = n(0)\exp(-\lambda_\kappa a). \qquad \text{(eq. 6.10)}$$

So the probability that the κ^{th} transition has occurred in a susceptible cell at age a will be

$$p_\kappa(a) = 1 - \exp(-\lambda_\kappa a). \qquad \text{(eq. 6.11)}$$

According to the model the transitions are independent. If all k transitions have occurred in a cell, then the cell is called a tumour cell. The probability that a tumour cell has arisen

$$p(a) = \prod_{\kappa=1}^{k}[1-\exp(-\lambda_\kappa a)]. \qquad \text{(eq. 6.12)}$$

The hazard $H(a)$ at age a is calculated according to eq. (6.3):

$$H(a) = \frac{n_s p(a-t)}{1-p(a-t)} \sum_{\kappa=1}^{k} \frac{\lambda_\kappa \exp[-\lambda_\kappa (a-t)]}{\{1-\exp[-\lambda_\kappa (a-t)]\}}. \qquad \text{(eq. 6.13)}$$

If all transition rates are equal, $\lambda_\kappa = \lambda$, then the hazard is given by

$$H(a) = \frac{n_s\, k\, \lambda \exp[-\lambda (a-t)]\, \{1-\exp[-\lambda (a-t)]\}^{k-1}}{1-\{1-\exp[-\lambda (a-t)]\}^{k}}. \qquad \text{(eq. 6.14)}$$

For a given number of susceptible cells and a constant lag time, the hazard decreases with decreasing transition rates and with an increasing number of necessary transitions (Fig. 6.2.). Under the assumption of $n_s = 10^9$ susceptible cells, in the two-step model rates of about 3×10^{-5} a^{-1} produce a cancer hazard that is comparable with observed cancer incidence rates. If more transitions are assumed to be necessary then the transition rates have to be considerably larger. This could be the case for epigenetic events, or if early mutations lead to an increase of later transition rates, e. g. by an induced genomic instability.

If for all transitions $\lambda_\kappa(a - t) << 1$, then the leading term in eq. (6.13) for $H(a)$ becomes

$$H(a) \approx n_s\, k\, (a-t)^{k-1} \prod_{\kappa=1}^{k} \lambda_\kappa. \qquad \text{(eq. 6.15)}$$

Nordling (1953) derived for male adults a proportionality of all cancer deaths to the sixth power of age, indicating in the frame of a simple multistage model that seven transitions would be necessary to create a cancer cell.

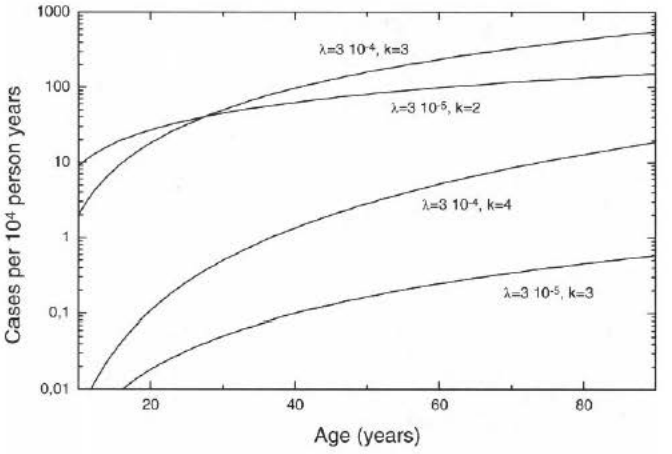

Fig. 6.2 Hazard as a function of age according to a multistep model of carcinogenis in which the sequence of cellular transitions is inconsequential (eq. 6.2.6); two transitions, each with $\lambda = 3 \times 10^{-5}$ yr^{-1}; three transitions, each with $\lambda = 3 \times 10^{-5}$ yr^{-1} or $\lambda = 3 \times 10^{-4}$ yr^{-1}; and four transitions, with $\lambda = 3 \times 10^{-4}$ yr^{-1}. The number of susceptible cells n_s has been assumed to be 10^9, and the lag time to be 5 years.

6.2.2 The Armitage-Doll model

Armitage and Doll (1954) proposed a model of carcinogenesis in which a susceptible cell has to undergo k events to become a cancer cell when the mutations occur in the right order (Fig. 6.3). Let $p_0(a)$ be the probability that a susceptible cell has not undergone the first mutation at age a, and $p_k(a)$ is the probability that a malignant cell develops at age a from a susceptible cell. According to the Armitage-Doll model, the probabilities $p_\kappa(a)$; $\kappa = 1,...,k$ that the cell has undergone the first κ mutations are described by the following system of differential equations:

$$\begin{aligned}
\dot{p}_0(a) &= -\lambda_1 p_0(a) \\
\dot{p}_1(a) &= \lambda_1 p_0(a) - \lambda_2 p_1(a) \\
\dot{p}_{k-1}(a) &= \lambda_{k-1} p_{k-2}(a) - \lambda_k p_{k-1}(a) \\
\dot{p}_k(a) &= \lambda_k p_{k-1}(a)\,,
\end{aligned} \qquad \text{(eq. 6.16)}$$

with the initial conditions $p_0(0) = 1$ and $p_\kappa(0) = 0$ for $\kappa = 1,..,k$. If all transition rates λ_κ are different and do not change over life time, then the solution of the system for

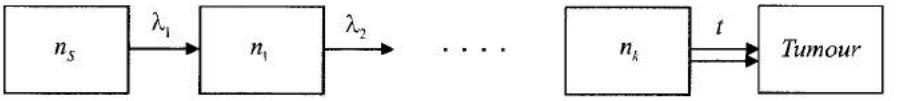

Fig. 6.3 The Armitage-Doll model. In order to become a cancer cell, susceptible cells have to undergo k transitions in the right order of occurrence. In addition, it is assumed here that the cancer cell needs a lag time t to develop to a detectable tumour.

$p_k(a)$ is a sum of exponentials of $\lambda_\kappa a$ with coefficients depending on λ_κ (see Moolgavkar 1978). It is straightforward to calculate the hazard according to eq. (6.3). If $\lambda_\kappa(a - t) << 1$ for all $\kappa = 1,...,k$, then the leading term for $H(a)$ becomes

$$H(a) \approx n_s (a-t)^{k-1} \left(\prod_{\kappa=1}^{k} \lambda_\kappa\right) / (k-1)!, \qquad \text{(eq. 6.17)}$$

which is apart from a factor k! the same as in the model in which the sequence of the mutations does not play any role (compare with eq. 6.15).

Armitage and Doll (1954) applied their model to the cancer mortality in different organs in England and Wales in 1950 and 1951. The mortality rates due to stomach, colon, rectum and pancreas cancer were proportional to the age to a power of 5 to 6.5. According to the model that would indicate that 6 to 7 or 8 cellular transitions would be necessary for a development of a healthy cell to a malignant cell. A second group of cancers – composed of cancer of lung, prostate, female breast, ovary and cervix and corpus uteri – did not follow the power law in eq. 6.17. Armitage and Doll attributed this to changes of endocrine secretions over lifetime which are considered to be important in these organs.

6.3 Models with clonal expansion

Clones of phenotypically changed cells as papillomas of the mouse skin, foci with altered enzyme activity in the rodent liver, or adenomatous polyps in the human colon are considered to be an intermediate state in the carcinogenic process. This indicates that intermediate cells have the ability to proliferate which has been introduced in models of carcinogenesis and is often called *clonal expansion*.

Multistage models with clonal expansion in general describe four main phases: (i) *Initiation* characterised by an accumulation of genetic changes leading to partial abrogation of growth control. (ii) *Promotion*, or clonal growth of the number of initiated or intermediate cells. (iii) *Malignant conversion* or *transformation*, which is an acquisition of further cellular changes leading to one (or more) malignant cells. (iv) *Tumour growth*, i. e. growth of the malignant cell to a detectable tumour or leading to the death of the organism. In general, carcinogens are considered to increase the transition rates of one or several of these phases.

6.3.1 The two-step clonal expansion model

One of the best analysed models of carcinogenesis is the stochastic two-step clonal expansion (TSCE) model (Moolgavkar and Venzon 1979). In this model, the promotion of intermediate cells is the net result of the stochastic processes of symmetrical cell division with rate α, death or differentiation with rate β, and asymmetric transformation with rate λ_2 (Fig. 6.4). In a first approximation, the promotion rate is equal to $\alpha - \beta$. The ratio β/α is the asymptotic probability of extinction of a clone (Luebeck et al. 2000). For $\beta > \alpha$ this probability is 1.

In the calculation of the hazard, clones of intermediate cells are followed until they have either died or until one of its cells has converted into a malignant cell. If

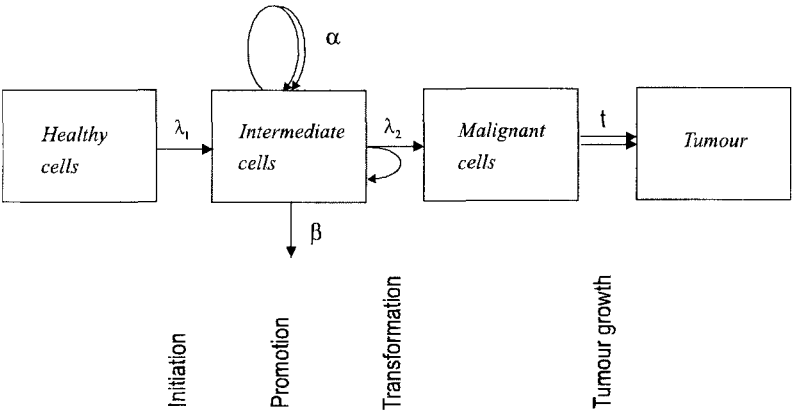

Fig. 6.4 Schematic presentation of the TSCE model with an initiation rate λ_1, and a division rate α, a death or differentiation rate β and a transformation rate λ_2 of intermediate cells[1]. During a lag time t a malignant cell is assumed to grow to a detectable tumour (if the model is adapted to incidence data) or to death due to the tumour (if the model is adapted to mortality data).

– for simplicity – the lag time is assumed to be a constant value t, then the hazard at an age a is proportional to the number of intermediate cells at the age a - t. Thus the assumption of a constant lag time facilitates the interpretation of model results. Analyses of the solid cancer incidence data among the atomic bomb survivors of Hiroshima and Nagasaki with constant lag times between zero and ten years gave similar results (Kai et al. 1997), indicating that the value of the lag-time or its distribution does not strongly influence the results. Also, Pierce and Mendelsohn (1999) obtained in simulation calculations for a multistep model of carcinogenesis, that there was little change of their results for the atomic bomb survivors if the standard deviation of the lag time is less or about 2–3 years. For simplicity, in this section a constant lag time t of five years is assumed.

6.3.1.1 The baseline hazard $H_0(a)$

In general, the solution of the stochastic two-step clonal expansion model needs complex mathematical tools (Moolgavkar et al. 1988; Moolgavkar and Luebeck 1990).[2] For model parameters that do not change over life time the model can be solved analytically (Kopp-Schneider et al. 1994; Zheng 1994). Such a model may be considered as the most simple approximation for spontaneous carcinogenesis. In this case the hazard $H_0(a)$ is completely determined by three combinations of the biological parameters (Heidenreich et al. 1996):

$$H_0(a) = \frac{X\ \{\exp[(\gamma+2q)(a-t)]-1\}}{q\ \{\exp[(\gamma+2q)(a-t)]+1\}+\gamma}, \qquad \text{(eq. 6.18)}$$

[1] In the literature, λ_1 or $(\lambda_1\ n_s)$ are often denoted by ν, and λ_2 by μ.

[2] In mathematical terms, the model can be described by a Markovian process in which the probability generating function satisfies the Kolmogorov forward differential equation.

with

$$X = n_s \lambda_1 \lambda_2$$

$$\gamma = \alpha - \beta - \lambda_2 \qquad \text{(eq. 6.19)}$$

$$q = (\sqrt{\gamma^2 + 4\alpha\lambda_2} - \gamma)/2 .$$

Such parameter combinations that determine the hazard are called identifiable parameters because in principle they can be determined from fits of the model to epidemiological or experimental data. In the parameter set in eq. 6.19, the parameter γ is sometimes called the effective clonal expansion rate[3], because for intermediate ages the number of initiated cells grows exponentially with the product of age (minus lag time) and the rate $\gamma + 2q$ (see below, eq. 6.21), and in most applications $2q$ is considerably smaller than γ.

Equation 6.18 was found to fit the age dependence of the baseline risk in radioepidemiological data well resulting in estimates of the parameters X, γ and q. Fig. 6.5 shows an example with the values $X = 10^{-6}$ a^{-2}, $\gamma = 0.13$ a^{-1} and $q = 3.5 \times 10^{-5}$ a^{-1} as they have been obtained for all solid cancers among the atomic bomb survivors from Hiroshima and Nagasaki with negligible radiation exposures (Jacob and Prokić 2003).

If two values of or relations between biological parameters are known or assumed, then estimates of the other biological parameters can be obtained. Assuming n_s equal to 4×10^8 and $\lambda_1 = \lambda_2$, one would obtain $\lambda_1 = \lambda_2 = 5 \times 10^{-8}$ a^{-1} and $\alpha \approx \beta \approx$ [illegible]$^{-1}$. Another possibility is to assume n_s equal to 10^8 and $\alpha = 9$ a^{-1}, resulting

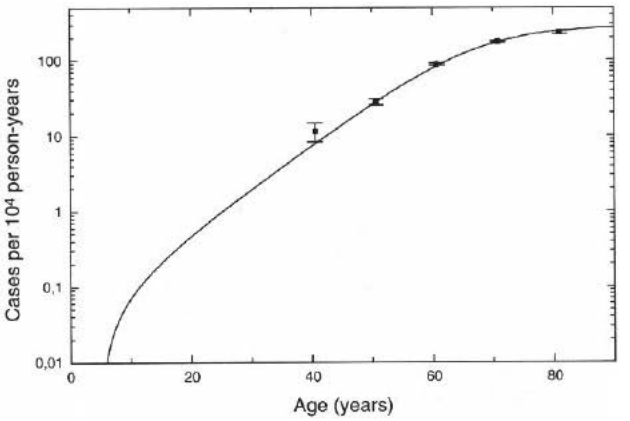

Fig. 6.5 Baseline hazard $H_0(a)$ for solid tumour incidence at age a among the male atomic bomb survivors with negligible radiation exposures, data points for the observation period 1958–1987 (black squares; Thompson et al. 1994), and as derived with the TSCE model (solid line; Jacob and Prokić 2003).

[3] A better approximation for the effective clonal expansion rate is, however, $\alpha - \beta + \mu \cdot (\alpha + \beta)/(\alpha - \beta)$.

in $\lambda_1 \approx 2 \times 10^{-8}$ a^{-1}, $\lambda_2 \approx 5 \times 10^{-7}$ a^{-1} and $\beta = 8.87$ a^{-1}. In this case, the mutation rate λ_2 of intermediate cells to malignant cells would be by about a factor of 25 larger than the mutation rate λ_1 of healthy cells to intermediate cells. In both cases, the proliferation rate α and the death or differentiation rate β of intermediate cells are similar, but their small difference is enough to cause a considerable exponential growth of the hazard over a large period of life. These numbers serve only as an illustration to what degree biological parameters can be determined. To obtain more meaningful conclusions, cancer-type specific estimates have to be made.

For young ages, the spontaneous hazard in eq 6.17 increases linearly with age:

$$H_0(a) \approx X(a - t); \qquad \text{for } a - t << (\gamma + 2q)^{-1}, \qquad \text{(eq. 6.20)}$$

as it would also result from the approximate solution of the Armitage-Doll model (eq. 6.17). In the numerical example given above, $(\gamma + 2q)^{-1}$ is about 8 years and the linear growth applies to an age period of 5 to about 9 years. In this phase the hazard growth with age is dominated by the initiation of susceptible cells to intermediate cells.

For intermediate ages, and as long as $\gamma/q >> \exp[(\gamma + 2q)(a - t)]$, $H_0(a)$ grows exponentially

$$H_0(a) = X \exp[(\gamma + 2q)(a - t)]/\gamma \qquad \text{for } a - t >> (\gamma + 2q)^{-1}$$
$$\text{and } a - t << \ln(\gamma/q)/(\gamma + 2q). \qquad \text{(eq. 6.21)}$$

In this phase, the hazard growth is dominated by the promotion of intermediate cells. The number of intermediate cells being produced in this age by initiating events becomes unimportant. In the example, this phase starts at an age of about 20 years. The value of $\ln(\gamma/q)/(\gamma + 2q)$ is about 60 years and the baseline hazard starts to deviate from the exponential growth at an age of about 40 years. In other examples, the period of the exponential growth of the hazard exceeds normal human lifetime.

For older ages, the exponential growth flattens and the hazard approaches an asymptotic value:

$$H_0(a) \Rightarrow X/q \qquad \text{for } a - t >> \ln(\gamma/q)/(\gamma + 2q). \qquad \text{(eq. 6.22)}$$

The existence of an asymptotic value is due to the fact that after a certain age clones have become so large that the sum of the probabilities for one cell within them being converted to a malignant cell and for an extinction of a clone is equal to the probability that a new clone is created by an initiating mutation.

6.3.1.2 Induced changes of parameters

Carcinogenic agents are assumed to change the parameters of the model for the time period of exposure, or changes may even persist after the time of exposure. An obvious example of a persisting effect is genomic instability. Another would be if parameters are changed during the period in which damage due to the exposure has to be repaired, either in the damaged cells or during the replacement of inactivated cells.

The effects of most exposure scenarios can be approximated by model parameters that are piecewise constant. Heidenreich et al. (1997b) showed that in this case

the model can be solved by a set of iterative equations and discussed various properties of the hazard after constant exposures over finite periods of time.

As shown in the example in Fig. 6.5, an exposure to an initiating agent during the age period of 40 to 50 years leads to a linear increase of the hazard in the age period of 45 to 55 years. Subsequently, the excess risk continues to increase due to the spontaneous promotion of the initiated cells.

In the case of a high exposure ($ERR = 2$) to a promoting agent, the hazard increases strongly during the first years after the age of 45. Subsequently, the increase becomes smaller. After the age of 55, the hazard decreases and even falls below the baseline hazard. These effects may be explained by the induced rapid growth of the number of intermediate cells in the clones. This increases the probability that a cell in the clone converts to a malignant cell. Since the corresponding organism is then considered to develop a cancer after a certain lag time, at later ages the whole clone is not contributing to the hazard anymore.

For lower exposures ($ERR = 0.2$) the same effects can be found, but are much less expressed. Here and in the following, exposures leading to an ERR of 0.2 are sometimes used to describe effects of low doses. However, it should be kept in mind, that an ERR of 0.2 for the incidence of solid tumours corresponds to an acute exposure of external ionising radiation with a dose of about 0.3 Sv (Thompson et al. 1994). This is a considerable dose and doses due to environmental contaminations will in general lead to smaller effects.

As noted in the previous subsection, for asymptotically large ages the spontaneous hazard approaches a constant level because an equilibrium is reached between the gain of new clones and the loss of clones in which a cell has become malignant or which are extinguished. Correspondingly, if the biological parameters return after an exposure to a carcinogen to the spontaneous levels, also the hazard will approach for longer times after the exposure to the spontaneous level (not shown in Fig. 6.5 because the depicted age intervals are too short). However, lifetime risk (eq. 6.5) will be always higher in the exposed group.

6.3.2 Multistep models with clonal expansion

Tan (1991) and Moolgavkar and Luebeck (1992) were the first to generalise the two-step clonal expansion model to multistep models of carcinogenesis with a clonal growth of intermediate cells. Little (1995) examined the behaviour of the excess risk for small instantaneous perturbations of the parameters in a number of generalisations of the TSCE model. More recent applications of multistep models with clonal expansion are described below and in section 6.4.

Colorectal cancer is a well studied example of multistep carcinogenesis (Fearon and Vogelstein 1990; Aaltonen et al. 1993; Peltomäki et al. 1993). Colorectal cancer is a main area of application of multistep models with clonal expansion to population-based data on cancer mortality (Herrero-Jimenez et al. 1998, 2000) and incidence (Moolgavkar and Luebeck 1992; Luebeck and Moolgavkar 2002). This section will deal exemplarily with the analysis of incidence data for colorectal cancer.

Luebeck and Moolgavkar (2002) developed a mathematical model for colorectal carcinogenesis based on the assumption that several genetic changes, e. g. the loss

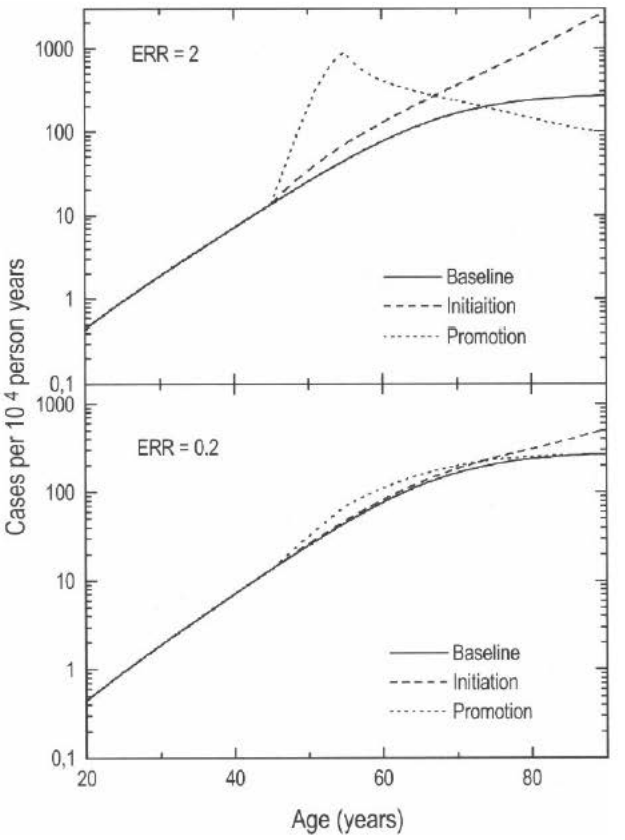

Fig. 6.6 Baseline hazard $H_0(a)$ to receive a solid tumor as obtained by an adaptation of the TSCE model to the solid tumour incidence data for the male atomic bomb survivors with negligible radiation exposures and total hazard after a constant exposure to an initiating and a promoting agent in the age period of 40 to 50 years. The exposures result in excess relative lifetime risks of 2 resp. 0.2 (Jacob and Prokić 2003).

or mutation of the tumour suppressing *APC* gene, precede the occurrence of clonally expanding adenomatous polyps in the colon. Correspondingly, they developed a series of multistep models (from the two-step (TSCE) to a five-step model), in which only cells that have undergone all but one changes in the carcinogenic process expand clonally by symmetric divisions (Fig. 6.6). They call these cells initiated, and those with less changes pre-initiated. Note, that for the pre-initiated cells division and death are not modelled explicitly. In the terminology used for the TSCE model, the initiated cells are called intermediate (Fig. 6.4).

For the application of the model to colorectal cancer incidence, it was assumed that there are 10^8 normal susceptible stem cells which is approximately the number of crypts in the colon. Incidence data for the period 1973–1996 and for nine geographic areas in the US Surveillance, Epidemiology and End Results (SEER) registry (Ries et al. 2001) were used. The data included about 256,000 colorectal can-

cer cases among the white population and 21,000 cases among the black population. Poisson regression was used to fit the models to the number of observed cases in the different age and calendar-year groups (see Appendix).

The four-step model was found to fit the data best. The two pre-initiating steps were interpreted as losses (or mutations) of the two alleles of the *APC* gene with transition rates of about 10^{-6} yr^{-1} (it was assumed that the two rates are equal).

The transition rate λ_3 of the initiating event was found to be of the same order of magnitude as the division rate α of initiated cells which is in the order of 10 yr^{-1} (Herrero-Jimenez et al. 1998), i. e. according to this model application the third transition is much more frequent than the pre-initiating events. Luebeck and Moolgavkar (2002) suggest the event to be an asymmetric division of the pre-initiated stem cell generating a progeny that moves from the stem cell zone into the proliferative zone of the crypt. Here, cell growth is not constrained by the microenvironment at the bottom of the crypt and the initiated cells can expand clonally via occasional symmetric cell divisions. Alternatively, the initiating step could be an epigenetic event.

The clones of initiated cells were interpreted to constitute adenomas and a growth rate α - β of the number of initiated cells of about 0.15 yr^{-1} was found. This corresponds to a doubling time of about 5 years. Based on the model results, Luebeck and Moolgavkar (2002) calculated the (age-dependent) number of these adenomatous clones for the healthy population and for individuals with familial adenomatous polyposis (FAP) who inherited a loss or mutation of one allele of the *APC* gene. Based on measurement data they estimated the number of aberrant crypt foci that are considered as the earliest stages of the formation of adenomatous polyps. The model data were by one order of magnitude lower, however, the model and the measurement-based estimation agreed well on the ratio of results for normal and FAP subjects.

As for the two pre-initiating events, the transition rate λ_4 for the initiated cells to convert to a malignant cell was found to be a rare event. The authors claim that the transition rate λ_4 is in the order of measured locus-specific mutation rates, indicating that there is no genomic instability in the carcinogenic process before the occurrence of this last rate-limiting step. They suggest that this rare event could be identified with mutation of one copy of the *p53* gene assuming a dominant negative action (Fearon and Vogelstein 1990; see also section 6.1.1). Alternatively, the occurrence of the cancer may be due to a combination of the rare mutation of one copy of the *p53* gene with the onset of genomic instability.

Multistep models of carcinogenesis should – at least in principle – allow a more realistic description of the carcinogenic process than the TSCE model. Taking into account the continuous growth of molecular genetic knowledge, it can be anticipated that multistep models will gain an increasing importance in the future.

6.4 Ionising radiation

Ionising radiation may be densely ionising, like α-particles and neutrons, or sparsely ionising, like X- and γ-rays. Densely ionising radiation has a considerably higher biological effectiveness per deposited energy, because it produces more complex DNA lesions per unit dose which have a higher probability of misrepair

(see chapter 3). The pathway of densely ionising radiation that is most relevant to the exposure of the population, is the inhalation of radon progeny (see chapter 5). It is also the pathway best analysed with models of carcinogenesis (section 6.4.1).

External exposure to sparsely ionising radiation is the most relevant pathway for many occupational exposures. The cohort of the atomic bomb survivors remains to be the most important information source for acute external exposures and has also been analysed with models of carcinogenesis (section 6.4.2). There is less information on effects of external exposures that occur with low dose rates over longer times.

At low doses of ionising radiation, a number of non-linear radiobiological effects have been observed. The model development to integrate such effects in models of carcinogenesis in order to analyse cancer risks at low dose is still in an early phase (section 6.4.3).

6.4.1 Effects of inhaled radon progeny

Large experiments have been performed in which rats were exposed to radon progeny in air. A main aim of the studies is an improvement of the understanding of time-, dose-, and dose-rate dependencies of the carcinogenic effect of radon exposures. An advantage of the animal experiments is a high degree of consistency in the control of exposures and in diagnosis of lung tumours. A disadvantage is that the results cannot be transferred without caution to humans, because in contrast to humans, rats can live with some frequent types of tumours for a long time.

In the mid of the 20th century, uranium miners were exposed to high concentrations of radon resulting in a significant increase of the lung cancer rate among them. Numerical estimates for lung cancer hazard were derived from corresponding cohort data. A common aim of the two approaches (animal experiments and epidemiological studies of miners) is to contribute to a better quantification of the radiation risk due to residential radon.

6.4.1.1 Animal experiments

Two main series of experiments were performed with radon exposures of rats, one at the Pacific Northwest National Laboratory (PNNL), Richland, WA (Cross et al. 1993) and one at the Commissariat à l'Energie Atomique (CEA), France (Moncheaux et al. 1999).

PNNL rats. In the experiments at PNNL, male SPF Wistar rats were exposed to constant radon levels for 18 h d^{-1} for 5 d wk^{-1}. The exposure lasted between 2 days and about 100 weeks (about 90 % of the average lifetime of the rat). At the beginning of exposure, the rats were 75 to 110 days old. In order to simulate the situation in mines, uranium ore dust was always administered with radon exposure.

In a first analysis of the PNNL data with a model of carcinogenesis, all lung tumours were treated as incidental, i. e. it was assumed that the tumours have not caused the death of the animal (Moolgavkar et al. 1990). In later analyses tumours were differentiated of being incidental or fatal (Luebeck et al. 1996; Heidenreich et al. 1999). In all three studies the data were analysed with the TSCE model.

Heidenreich et al. (1999) used a data set for 4,276 rats. Of these 3,726 lived out their life span, 418 were sacrificed according to a planned schedule, 127 were euthanised for ethical reasons, and 5 were killed accidentally. A total of 487 rats developed at least one lung tumour. Based on the constant parameters for the TSCE model in eq. 6.18, the following dependencies on the average exposure rate d (accumulated exposure divided by time interval from beginning of first fraction of exposure until end of last fraction of exposure) were introduced:

$$\begin{aligned} X(d) &= n_s\, \lambda_1(d)\, \lambda_2(0) \\ \gamma(d) &= \alpha(d) - \beta(d) - \lambda_2(d) \\ m(d) &= \lambda_2(d)/\lambda_2(0) \\ q(d) &= q(0). \end{aligned} \qquad \text{(eq. 6.23)}$$

$\lambda_1(d)$, $\gamma(d)$ and $m(d)$ were assumed to increase linearly with radon exposure rate, where additionally the initiating mutation rate $\lambda_1(d)$ had an exponential cell killing term for high doses, and the clonal expansion rate $\gamma(d)$ levelled off to a constant term at higher doses. Individual likelihood techniques (see Appendix) were used to fit the model to the data. Age and exposure patterns of the hazard for incidental and fatal lung tumour in rats were found to be quite different.

For incidental lung tumours in rats there is, according to the model fit, a large spontaneous initiation rate for intermediate cells. The initiation rate increases linearly with exposure rate. The main radiation effect, however, is to increase the size of clones of intermediate cells by an increased proliferation rate. The exposure-rate dependence of this promotion effect was found to be nearly a step function, i. e. small exposure rates have an effect that is comparable to high exposure rates. This might be due to the presence of uranium dust in the air for all radon exposures. The effect of the radiation on the second mutation rate was not significant.

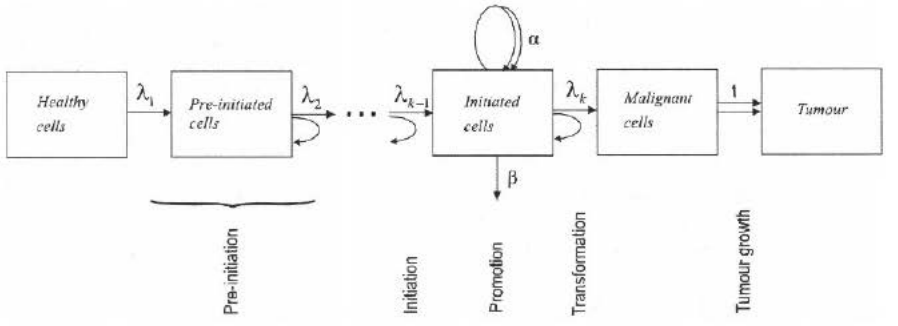

Fig. 6.7 k-step models of the carcinogenic process in which (k - 1) cellular changes are necessary to produce initiated cells that can undergo clonal proliferation (after Luebeck and Moolgavkar 2002)[2]

[4] Luebeck and Moolgavkar (2002) denote λ_K by μ_{K-1}.

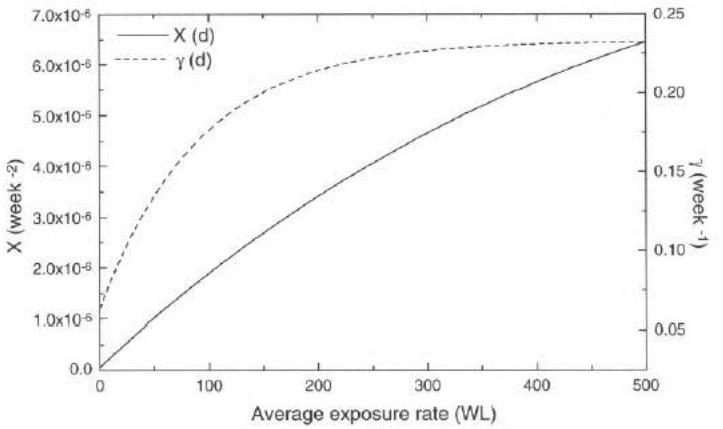

Fig. 6.8 Exposure-rate dependence of TSCE model parameters X (proportional to the initiation rate) and γ (effective promotion rate) for fatal lung cancer among radon exposed rats (after Heidenreich et al. 1999)

Concerning fatal lung tumours, the spontaneous initiation rate for intermediate cells was found to be small, the spontaneous promotion rate to be quite large. Radiation effects on both, initiation and promotion rate, turned out to be relevant for fatal tumours. For both rates, low radon concentrations had an higher effect per unit exposure than higher concentrations (Fig. 6.7). As for incidental tumours, the effect of the radiation on the second mutation rate was not significant.

Heidenreich et al. (1999) studied the excess relative risk per unit radon exposure (ERR WLM^{-1})[3] for fatal lung tumours at an average lifetime for rats exposed from an age of 10 weeks with different exposure rates until the requested exposure was applied (Fig. 6.8). According to the model fit, there is an inverse exposure-rate effect for high exposure rates which is especially expressed for high exposures. There are two reasons for the inverse dose rate effect. Firstly, for high exposure rates the radiation induced promotion is of large importance. Lower exposure rates last longer so that in this exposure rate range the radiation induced promotion acts in average on a larger number of spontaneously initiated and promoted cells (high dose rate protraction effect). Secondly, due to the curvature of the exposure rate dependence of the promotion, lower exposure rates with longer exposure times cause a higher promotion than high exposure rates with short exposure times (Fig. 6.7). The inverse dose rate effect for high exposures was also found by an analysis of the data with an excess relative risk model (Gilbert et al. 1996).

For low exposure rates, the model predicts a direct dose-rate effect (see Fig. 6.8). This is because for lower exposure rates in this exposure rate range, the radiation-induced intermediate cells have in average less time to proliferate due to the spontaneous promotion (low dose rate protraction effect). This effect is also found in situations in which radiation acts dominantly on initiation (Moolgavkar 1997). It should, however, be noted that the low dose rate protraction effect (spontaneous promotion

[5] For a definition of the unit WLM see Section 3.5.1 or the glossary.

of radiation-induced intermediate cells) predicts an inverse dose rate effect for protracted exposures that end at the same time (not start at the same time).

Two points are worth noting concerning the protraction effects. Firstly, both effects occur in the discussed exposure scenarios, because the low dose rate exposures extend to a later period of life. Secondly, the dose rate effect (low dose rate protraction effect) is due to the age-at-exposure dependence in TSCE models with a non-negligible radiation effect on the initiation rate (see also section 6.4.3). A dose-rate dependence of DNA damage repair has not been taken into account in these versions of the TSCE model.

Besides the dose rate effects, two aspects of extrapolating from high doses to low doses can be observed for the model fit and the exposure scenarios. For high exposure rates, the excess relative risk per unit exposure is larger for high exposures than for low exposures (Fig. 6.8). This is again due to the high dose rate protraction effect. The radiation-induced promotion is important in this exposure rate range. Higher exposures last until higher ages, and therefore, in average the radiation-induced promotion acts on more spontaneously initiated and promoted cells. For low exposure rates the opposite trend is found due to the low dose rate protraction effect.

CEA rats. At CEA, male Sprague-Dawley rats were exposed to radon and/or various chemicals (Monchaux et al. 1994). Three groups of modellers analysed commonly a CEA data set of lung cancer among 3,503 rats that were exposed to radon and its progeny only and among 1,525 that served as controls (Heidenreich et al. 2000). For 3,342 of the rats the exposures started at an age of 3 months, exposure durations varied between 0.5 months and 16.4months, the cumulative exposures between 25 and 10,000 WLM. For 161 rats, the start of the exposure was in the age period 9 to 15 months. Of the rats 406 had at least one lung tumour. All tumours were treated as incidental. Individual likelihood techniques were used to fit models of carcinogenesis to the data. Using a simple test model, the authors showed that their mathematical approaches and fit routines gave comparable results. Subsequently, they analysed the data set with their own models of carcinogenesis. An overview over the models preferred by the three groups is given in Tab. 6.1.

The first group of modellers favoured a TSCE model with an action on promotion and did not find an effect on the second mutation rate, as for the application to the PNNL rats. Only identifiable parameters were used and a value for the lag time t was fitted. The model was optimised by assuming a constant value of the radiation inducing the promotion rate above a threshold of 1 WL[4] and below an exposure rate value of 250 WL. It has not been explained why the promoting action should disappear for higher exposure rates.

The second group favoured a TSCE model in which the two spontaneous mutation rates were set equal. They assumed linear exposure rate dependences of the mutation rates with exponential cell killing terms that also apply to the spontaneous mutation rate. The stochastics of the birth-death process were not found to be relevant in their approach, therefore only one parameter for the exponential growth of the number of intermediate cells was assumed. It was not tested whether the radiation had an effect on the promotion of intermediate cells. A number of 5×10^5 susceptible normal cells for lung tumours in rats was assumed.

[6] For a definition of the unit WL see Section 3.5.1 or the glossary.

Tab. 6.1 Parameters in models of carcinogenesis that were used by three groups of modellers in analysing the CEA data on lung cancer among radon exposed rats, p_i are the fit parameters (after Heidenreich et al. 2000).

TSCE model of first group	**TSCE model of second group**
$X = p_1 (1 + p_2 d)$	$\lambda_1 = (p_1 + p_2 d) \exp(-p_3 d)$
$\gamma = p_3 + p_4 \Theta(1\text{ WL}; 250\text{ WL})$[a]	$\lambda_2 = (p_1 + p_4 d) \exp(-p_5 d)$
$q = p_5$	$\alpha - \beta = p_6$
$t = p_6$	$t = p_7$
TSCE model of third group	**Three-stage model of third group**
$\lambda_1 = p_1 + p_2 \cdot d$	$\lambda_1 = p_1 + p_2 d$
$\lambda_2 = p_1$	$\lambda_2 = p_1$
$\alpha - \beta = p_3 + p_4 (1 - \exp(-p_5 d))$	$\lambda_3 = p_1 + p_3 d \exp(-p_4 d)$
$T = p_6$[b]	$T = p_5$[b]
$n_{s+} = p_7$[b]	$n_{s+} = p_6$[b]

[a] $\Theta(1\text{ WL}; 250\text{ WL})$ is 1 for the exposure rate range of 1 to 250 WL and zero otherwise

[b] It is assumed that after age T the number n_s (5×10^5) of susceptible healthy cells is changed to n_{s+}

The third group favoured two models, a TSCE model and a three-step model. In both models, the number of susceptible normal cells was allowed to change from 5×10^5 to another constant value at an age that is also a fit parameter. The spontaneous mutation rates were assumed to be equal. A linear exposure rate dependence of the first mutation rate was assumed. The death or differentiation rate of intermediate cells was set to zero. In the TSCE model, a linear increase of the proliferation rate with exposure rate was assumed, that levels off exponentially to a constant value. In the preferred three-step model clonal expansion was not taken into account at all, and the third mutation rate was assumed to increase linearly with exposure rate with an exponential cell killing term.

All four models were considered to describe the data well. In all four models the exposure rate dependence of the initiation rate for intermediate cells turned out to be similar. The ratio $\lambda_1(d)/\lambda_1(0)$ increases linearly (in one case with a slight downward bending due to the cell killing term) up to a value of 650–850 for exposure rates of 500 WL.

The radiation effect on the first mutation rate alone was not found to be sufficient for a satisfactory fit. The agreement with the data could be improved by assuming a radiation effect on the promotion rate or on the last mutation step. No further improvement was observed when both were increased. The three groups obtained comparably good fits by modelling radiation effects on promotion and last mutation rates quite differently.

The spontaneous hazard for lung cancer among radon exposed rats is modelled similarly by the four models for the whole life span with two exceptions (Fig. 6.9). The first group obtains lower spontaneous hazards at older ages, deviating by a fac-

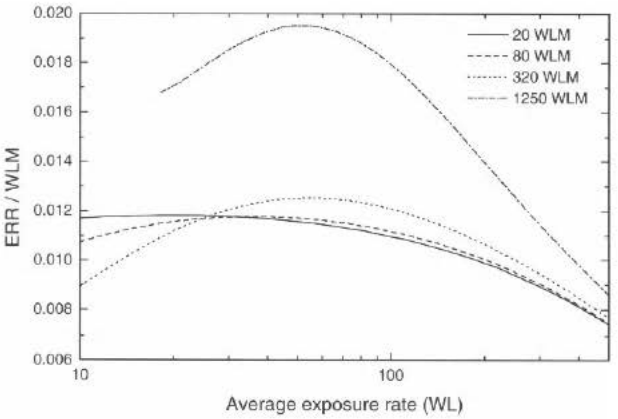

Fig. 6.9 Excess relative risk per unit radon exposure (ERR WLM^{-1}) for fatal lung tumours for rats with an age of 110 weeks having been exposed since an age of 10 weeks. Results are given according to an TSCE model application to the PNNL data (after Heidenreich et al. 1999).

tor of 2 from the other models at an age of 150 weeks. The three-step model gives considerably lower hazards than the TSCE models for ages younger than 30 weeks. It should be noted that the number of cases in the data set outside the age range of 30 to 150 weeks is small.

For the example of an exposure during an age period of 13 to 33 weeks with an exposure rate of 50 WL, the four models agree on the hazard within a factor of 3 only in the age period of 57 to 100 weeks. For older ages, the first group obtains considerably lower hazards. For younger ages, the TSCE models of the first two groups agree quite well, and so do the TSCE and three-step model of the third group.

In summary, the exercise of the three modeller groups has clearly demonstrated that a variety of models is equally well compatible with the data. Besides the quality of fit, plausibility and radiobiological knowledge are further criteria to support or rule out possible models. Independent of this, the application of a variety of plausible models is a possibility to explore model uncertainties and identify results that are obtained with all of the models.

6.4.1.2 Uranium miners

Moolgavkar et al. (1993) used the TSCE model to analyse the lung cancer mortality among miners who worked in the Colorado Plateau uranium mines between 1950 and 1964. In a more recent analysis (Luebeck et al. 1999), data with an extended follow-up (up to the end of 1990) were used. Detailed patterns of exposure to radon and cigarette smoke were simulated, and exposures from prior hard rock mining were taken into account. Data on 3,347 white male miners were analysed. For each of the miners times where given when their cumulative exposure exceeded 60, 120, 360, 600, 840, 1,800 and 3,720 WLM. For cigarette smoking, up to five times were

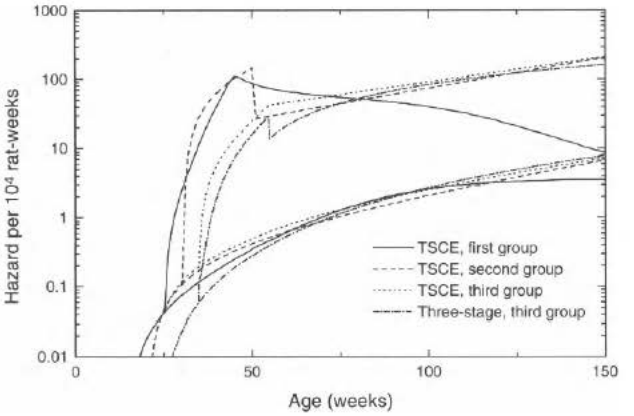

Fig. 6.10 Spontaneous and total hazard among male Sprague-Dawley rats exposed at CEA to radon in an age period of 13 to 33 weeks with an exposure rate of 50 WL. Results are given according to three TSCE model versions and a three-step model (after Heidenreich et al. 2000).

given when smoking levels (in packs d^{-1}) changed. For each miner birth year, age at entry into the study (first medical examination), attained age, and vital status were given. If the miner died before the end of the study, the listed ICD code indicated his cause of death and 354 of the miners died because of lung cancer with the ICD code 162 (malignant neoplasms of trachea, bronchus and lung).

Luebeck et al. (1999) used the TSCE model version defined in eq. 6.23 to analyse the data. They assumed that the first mutation rate increases linearly with birth year and mean radon exposure, and with smoking rate according to exp[1 - const· (smoking rate squared)], see Fig. 6.10. Although detailed data on the smoking behaviour were taken into account, an unexpected strong dependence of the initiation rate on the birth year was found. This may be related to problems with the smoking data. According to the data set, for instance, miners below age 70 had fewer lung cancer cases in the highest smoking group compared to miners belonging to the second highest smoking group.

In the model application, the clonal expansion rate was assumed to increase logarithmically with mean radon exposure rate and was found to be only slightly larger for smokers than for non-smokers. In the preferred model, the second mutation rate is independent of smoking and mean radon exposure rate. The lag time t was estimated to be about 9 years. All parameter estimates (except for the spontaneous promotion rate) have a large uncertainty and no information is given on correlations. Therefore, all conclusions on age, time and exposure dependences have also large uncertainties.

For exposures centred around the age 40, the excess absolute lifetime risk (up to age 70, other causes of death neglected) per unit exposure is found to first increase with duration of exposure, reach a maximum and then decline (Fig. 6.12). Thus an inverse dose rate effect is found for short exposure times, and a direct dose rate effect for long exposure times. The lower the total exposure, the weaker the inverse dose rate effect and the sooner the direct dose rate effect sets on.

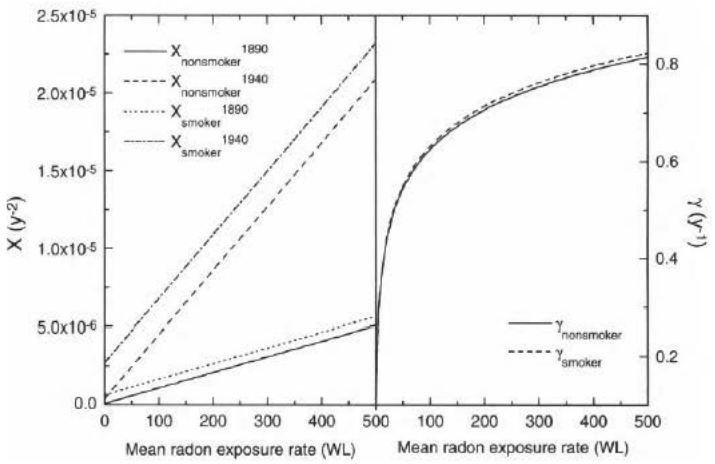

Fig. 6.11 Parameters of the TSCE model as assessed by Luebeck et al. (1999) for the Colorado Plateau miners. For smokers a smoking rate of 10 cigarettes d^{-1} has been assumed, the birth year is indicated in the legend by the parameter X. The estimates have large uncertainties.

As for the exposure pattern discussed for the data for the PNNL rats (section 6.4.1.1), the inverse dose rate effect is found for high exposure rates and the direct dose rate effect for low exposure rates (Tab. 6.2). In the scenario for the miners, the inverse dose rate effect is mainly caused by the downward curvature of $\gamma(d)$. The direct dose rate effect is due to the strong promotion of the initiated cells in the intermediate time interval which is not fully compensated by a weaker promotion over a longer time interval. Radiation induced initiation plays only a negligible role for the parameters and ages considered here.

For exposures with high exposure rates centred around the age 40, the excess absolute lifetime risk is higher for low exposures than that for high exposures (Fig.

Tab. 6.2 Dose and dose-rate effects for lung cancer risks due to protracted exposures to radon.

Exposure rate	Excess relative risk for rats at age of 110 days for exposures starting in young adult age (after Heidenreich et al. 1999)	Excess lifetime absolute risk up to age 70 for humans with exposures centred around age 40 (after Luebeck et al. 1999)
High	Excess risk per unit dose decreases with decreasing dose; *Inverse* dose rate effect	Excess risk per unit dose increases with decreasing dose; *Inverse* dose rate effect
Low	Excess risk per unit dose increases with decreasing dose; *Direct* dose rate effect	Excess risk per unit dose decreases with decreasing dose[a]; *Direct* dose rate effect

[a] For exposures smaller than 200 WLM

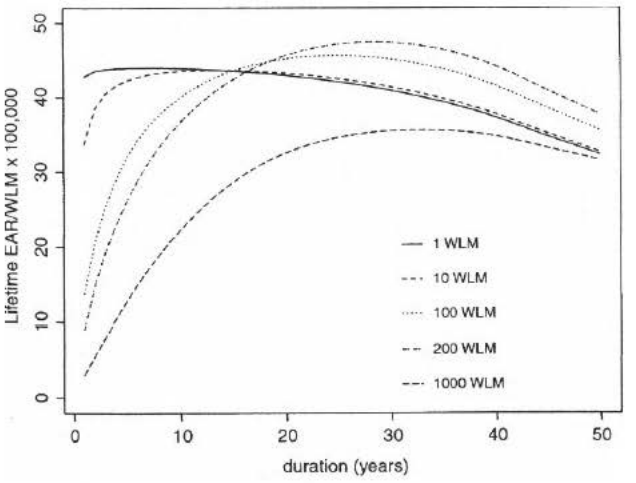

Fig. 6.12 Excess absolute lifetime risk WLM^{-1} (up to age 70, competing risks are neglected) after exposures to radon that are centred around age 40, as derived with the TSCE model for smokers among the Colorado Plateau miners (from Luebeck et al. 1999). A smoking rate of 10 cigarettes d^{-1} starting at age 15 has been assumed.

6.11). For low exposure rates, the inverse is found (Tab. 6.2). These trends are opposite to what has been observed in the exposure scenario for the PNNL rats (Fig. 6.8).

It may be noted that in contrast to the exposure scenarios shown in Tab. 6.2, for protracted exposures with low exposure rates that end in the same age, in most cases an inverse dose rate effect is predicted. It may be concluded that for protracted exposures the excess risk per unit dose depends in a complex manner on dose, dose rate, exposure pattern and action mechanisms of the radiation on the carcinogenic process. Correspondingly, the discussed applications of models of carcinogenesis do not contribute further evidence to the assumption that the LNT extrapolation does not lead to an underestimation of the risk at low doses and dose rates.

6.4.2 Effects of external exposures

6.4.2.1 Atomic bomb survivors

The data on solid cancer incidence among the atomic bomb survivors from Hiroshima and Nagasaki (Thompson et al. 1994) are the radioepidemiological data most frequently analysed with models of carcinogenesis. Soon after the data became available, work was published on two analyses of the data with the TSCE model assuming that radiation acts only in the first mutation rate (Heidenreich et al. 1997a; Kai et al. 1997). Later, the data were analysed with multistage models (Pierce and Mendelsohn 1999), which was then compared with results obtained by the TSCE model (Heidenreich et al. 2002). The data were also used to explore pos-

sible effects of low-dose hypersensitivity on lung cancer risk estimates (section 6.4.3).

The data cover the observation period 1958-1987. They are stratified in age-at-exposure categories (five years intervals up to 60 and older than 60), attained age (five year intervals), and colon dose equivalents D (cut points 0.005, 0.01, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 Sv).

Most of the analyses were performed with TSCE models in which the γ-radiation acts only as an initiator and causes instantaneously an increase of the initiation rate that is proportional to the dose:

$$\lambda_1(a, D) = \lambda_1(0) + c_1 D \delta(a - a_e), \qquad \text{(eq. 6.24)}$$

where a_e is the age at exposure and $\delta(a)$ the Dirac δ-distribution. Alterations of the second mutation rate were not considered because resulting cancer cases would have been probably observed before 1958 and therefore not have been included in the data. Under these conditions, the hazard $H(a, D)$ can be calculated analytically (Heidenreich et al. 1997a):

$$H(a, D) = H_0(a) + \frac{c_1 \mu_2 n_s D (\gamma+2q)^2 \exp[(\gamma+2q)(a-a_e-t)]}{\{\gamma+q+q\exp[(\gamma+2q)(a-a_e-t)]\}^2}, \qquad \text{(eq. 6.25)}$$

where $H_0(a)$ is the spontaneous hazard (eq. 6.18).

Kai et al. (1997) analysed the data for solid cancer in lung, stomach and colon. Poisson regression techniques were applied to fit the models to the data. A variation of the lag time t in the range of 0 to 10 years made little difference in the results. Kai et al. tested various dependences of the model parameters on birth year which is in the case of an acute exposure directly related to the age at exposure. In the preferred models of Kai et al. (1997), the spontaneous initiation rate depends linearly on birth year (only for stomach cancer among males no significant birth year effect was found). The spontaneous lung and colon cancer incidence at a given age were found to increase with birth year. On the contrary, stomach cancer incidence was found to decrease with birth year (only for females). These trends are in agreement with data from the tumour registry in Hiroshima.

Tab. 6.3 Ratios of the radiation induced initiation rate $\Delta\lambda_1(D)$ to the spontaneous initiation rate $\lambda_1(0)$ for organ equivalent doses of 1 Sv, as derived by Kai et al. (1997) with the TSCE model from the cancer incidence data among the atomic bomb survivors with different ages at exposure (a_e).

Organ	Sex	$\Delta\lambda_1(1\text{ Sv})/\lambda_1(0)$		
		$a_e = 5$	$a_e = 30$	$a_e = 45$
Lung	Male	11	15	19
	Female	25	208	804
Stomach	Male	8	8	8
	Female	53	28	22
Colon	Male	39	66	115
	Female	41	66	103

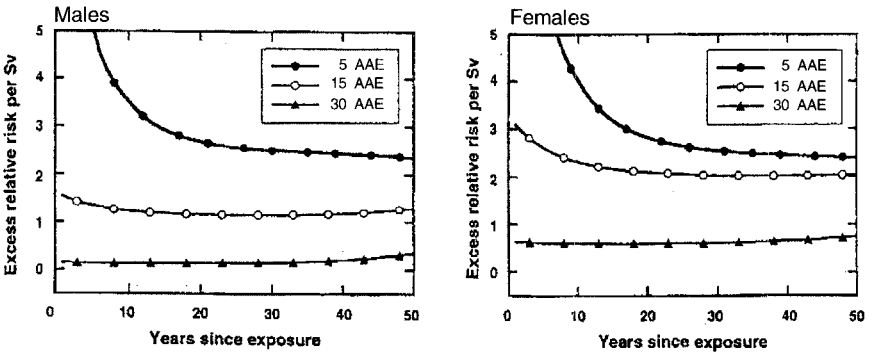

Fig. 6.13 Excess relative risk per unit dose to obtain colon cancer after an acute exposure to external radiation, as assessed with a TSCE model from the atomic bomb survivors data (from Kai et al. 1997).

In general, Kai et al. (1997) found that the radiation induced initiation is independent of birth year (only for lung cancer among females a birth-year dependence was found). Correspondingly, the ratio of the radiation induced initiation rate $\Delta\lambda_1(D)$ to the spontaneous initiation rate $\lambda_1(0)$ increased with age at exposure, with the exception of stomach cancer (Tab. 6.3). The authors argue that these ratios are broadly consistent with ratios that can be estimated from experimental radiobiological data for the *Hprt* gene and for the *GPA* gene. For lung cancer among females that were adult at the time of exposure, however, the values for the initiation ratio in Tab. 6.3 are exceptionally high, which might be due to a correlation of smoking and dose among female survivors in Hiroshima (Preston 1999).

Kai et al. (1997) found that the *ERR* decreases with age at exposure (Fig. 6.13). This is because the number of radiation induced intermediate cells are assumed to be independent of age and the number of spontaneously initiated intermediate cells increases with age. For most cancer sites a decrease of the *ERR* with age at exposure was also found by analyses of the data with conventional constant excess relative risk models (Thompson et al. 1994). However, in some cases the trend is inversed leading to contradictions of the two models. For lung cancer among males, e. g. Kai et al. (1997) found the excess relative lifetime risk for lung cancer (up to an age of 70) to decrease with age at exposure from 5 to 45 by a factor of 280, whereas the authors found for the ICRP-1990 model a decrease by a factor of 4 and for the BEIR V model an increase by a factor of 4.

For young ages at exposure, the *ERR* decreases according to the analysis of Kai et al. (1997) for all three cancer types analysed within the first decade after exposure, subsequently approaching a constant value. Again this is because the number of spontaneously initiated cells increases with age which is important for young age and only a minor relative increase in larger age.

For older ages at exposure, the *ERR* is essentially constant up to 40 years after exposure, because in this age range the growth of the number of intermediate cells is determined by the proliferation of intermediate cells. For even longer periods after exposure, the *ERR* is predicted to increase because the flattening of the exponential increase of the hazard at high ages comes later for the clones of intermediate cells

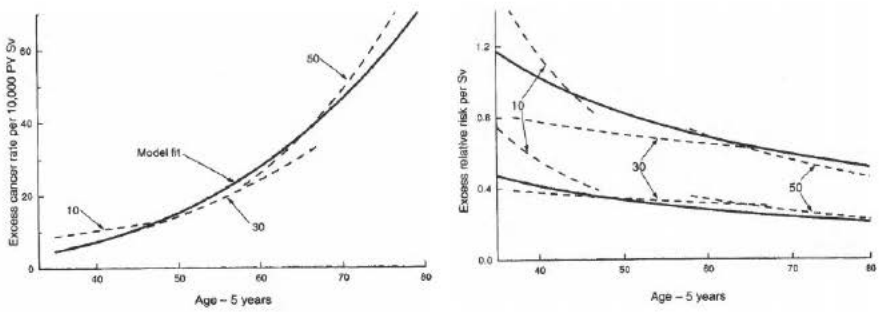

Fig. 6.14 Excess absolute risk (left panel, both sexes pooled) and excess relative risk (right panel, upper curves for females and lower curves for males) for solid cancer (excluding thyroid cancer and sex-specific cancers) among the atomic bomb survivors. The solid lines represent a fitted multistage model, the dashed lines empirical descriptions of the data. The numbers at the curves indicate the age at exposure (from Pierce and Mendelsohn 1999).

that were initiated by radiation (Fig. 6.6). It may be possible to test this prediction by the upcoming data for a longer follow-up. It may be noted that the increase of the *ERR* at long times after the exposure is a characteristic of TSCE models, in which the radiation acts dominantly on the initiation rate. It is less expressed or even vanishes if the radiation acts dominantly on the promotion rate (Fig. 6.6).

Age-time patterns of excess risks for solid cancer incidence in organs that are not strongly influenced by hormones, i. e. excluding thyroid cancer and all sex-specific cancers, have been analysed by Pierce and Mendelsohn (1999) with Armitage-Doll type multistep models (Fig. 6.3). The main aim of the study was to identify models of carcinogenesis explaining that the excess absolute risk mainly depends on age attained. The authors could explain main age-time characteristics of the excess risk without assuming additional dependences on age at exposure or time since exposure. They show that the approximate a^{k-1}-dependence of the spontaneous hazard in the k-step model also holds, if there are some restrictions on the order of the mutations, and if mutations can substantially alter the rate of subsequent ones.

Pierce and Mendelsohn (1999) assumed that radiation can act on any of the mutation rates and that the radiation action on the mutation rate does not depend on the age a. They claim that then for k being not too large, the excess hazard has an approximate a^{k-2} dependence. This implies that the excess relative risk decreases with age proportionally to a^{-1}. The fit of the model to the solid tumour incidence results in a value of k of 5.3. In general, the model fits well the age dependence of the excess hazard and of the relative risk of solid cancer among the atomic bomb survivors (Fig. 6.14). However, the agreement is not that good for cohort members who were exposed during childhood.

Stimulated by the work of Pierce and Mendelsohn (1999), Heidenreich et al. (2002) published a comparative analysis of the incidence data among the atomic bomb survivors with the TSCE model and with multistage models of carcinogenesis. In the case of the TSCE model, it was assumed that the radiation acts only on the initiation rate, and in the multistage model on any one of the k mutations. Heidenreich et al. (2002) used exact solutions of the multistage model in which the sequence of cellular transitions is inconsequential and of the Armitage-Doll model (section 6.2).

Numerically, all models fitted the data comparably well. So, none of the different models could be ruled out due to statistical reasons. However, in contrast to the approximate solutions used by Pierce and Mendelsohn (1999), the exact solutions of the multistep models gave implausible results for the number k of steps in the model. For the selection of solid tumours analysed by Pierce and Mendelsohn (1999), e. g. the estimated number of steps is for males (about 15) by a factor of 3 larger than for females (5–6). Similar discrepancies were found for single cancer sites analysed (lung, colon and stomach). Heidenreich et al. (2002) argue that the reason for this discrepancy is that the exact solutions of the multistep model without clonal expansion of intermediate cells exhibit an unrealistic form of levelling-off of the hazard for older ages and suggest that models with clonal expansion should be used instead.

Mortality data for the atomic bomb survivors have also been analysed with multistage models and the interested reader is referred to Little (1996).

6.4.2.2 Threshold considerations

Prokić and Jacob (unpublished) applied TSCE models with and without a threshold to the solid cancer incidence (Thompson et al. 1994) and mortality (Pierce et al. 1996) data among the atomic bomb survivors from Hiroshima and Nagasaki. The radiation was assumed to act instantaneously on the first mutation rate and eq. 6.24 was replaced by

$$\lambda_1(D) = \lambda_1(0) + c_1 (D - D_t)\, \Theta(D_t)\, \delta(a - a_e), \qquad \text{(eq. 6.26)}$$

where D_t is the threshold dose and $\Theta(D_t)$ is zero for $D < D_t$ and 1.0 otherwise. Calculations were performed for the following values for D_c: 0 (no threshold),10, 20, 50, 100, 150, 200, 300, 400 and 500 mSv.

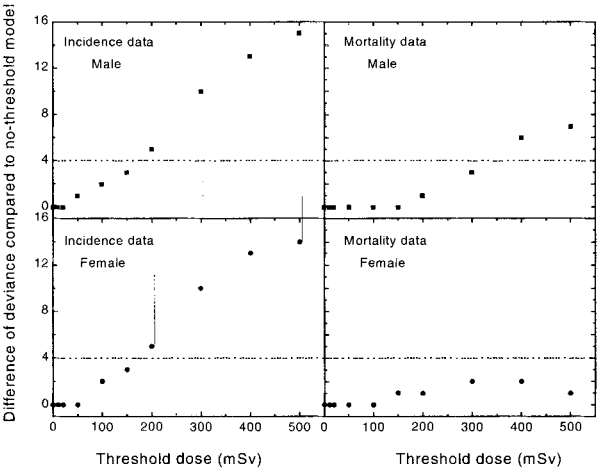

Fig. 6.15 Differences of deviance when fitting TSCE models to the cancer incidence and mortality data for the atomic bomb survivors from Hiroshima and Nagasaki. Models with a linear action of the radiation on the first mutation rate with and without a thresholds have been used (from Prokić and Jacob 2002).

Poisson regression was performed to fit the model to the data. The deviance was used as a measure of the quality of the fit, a lower deviance indicating a better fit. The deviance for the model without threshold is subtracted from the deviance for the models with threshold (Fig. 6.15). Negative values would indicate that the threshold model fits the data better. Positive values exceeding 4 were considered to indicate that the model is not supported by the data (see Appendix).

The deviance for the threshold models was found in no case to be smaller than the deviance of the no-threshold model. So, none of the considered threshold models fits the data better than the no-threshold model. For the cancer incidence among males and among females, the difference in deviance exceeds the value of 4 for threshold doses larger than 200 solid tumour incidence among the atomic bomb survivors cannot exclude the existence of a threshold in the dose range of 0 to 200 mSv. In the case of mortality data, fits for males can not exclude the existence of a threshold below 400 mSv; data on females are not at all conclusive on the existence of a threshold.

6.4.3 Low-dose radiobiological effects and low-dose risk estimation

Radiobiological experiments have revealed various effects at low doses which exhibit a non-linear dose dependence, e. g. adaptive response, low-dose hypersensitivity, bystander effects and genomic instability (see chapter 3). Up to now, there is only very limited work on exploring possible low-dose implications of these effects with the help of models of carcinogenesis. Some work has been performed on the application of a TSCE model incorporating low-dose hypersensitivity for lung cancer incidence among the atomic bomb survivors from Hiroshima and Nagasaki (Jacob and Prokić 2002; Prokić et al. 2002). Earlier applications of the TSCE model (section 6.4.2.1) had problems with the age-at-exposure dependence of the *ERR*.

Low-dose hypersensitivity. A number of radiobiological studies have indicated the presence of a hypersensitive region in the dose dependence of the survival response of many cell lines after exposure to low-LET radiation (Joiner et al. 1996, 2001). For most cell lines the number of inactivated cells per unit dose is higher for low doses (up to a few hundred mGy) than for high doses where an increase in radioresistance (IRR) is observed. Low-dose hypersensitivity in cell inactivation has also been observed *in vivo* for rhabdomyosarcoma R1H in rats (Beck-Bornholdt et al. 1989) and for normal human skin cells that were irradiated off the centre of the beam during radiotherapy of prostate cancer patients (Joiner et al. 2001).

The dose dependence of cell inactivation after exposures to low-LET radiation is modelled by survival curves that account for increased radioresistance (IRR)

$$S(D) = \exp[-a_r(1+(a_s / a_r - 1)\exp(-D / D_c))D - bD^2], \qquad \text{(eq. 6.27)}$$

where D is dose, a_r and a_s are the coefficients of the linear dose term in the high-dose (IRR) and low-dose regime, respectively, b is the coefficient of the quadratic dose term and D_c is the dose characteristic for the transition from the low-dose to the high-dose regime. An increased radioresistance at higher doses is expressed by $a_r < a_s$. For normal human lung epithelial cells L132 Singh et al. (1994) obtained the

values $a_r = 0.15\ Gy^{-1}$, $a_s = 1.19\ Gy^{-1}$, $D_c = 0.58$ Gy and $b = 0.07\ Gy^{-2}$ by fitting the function in eq. 6.27 to their experimental cell survival data.

Promoting effect of cell inactivation. It has been proposed that cell inactivation by carcinogens may cause a promotion, i. e. an increase of the number of intermediate cells (Trosko et al. 1983; UNSCEAR 2000). The suggested reason is that the inactivation of normal cells reduces the suppression of the proliferation of intermediate cells. Another possible reason is that intermediate cells have a higher potential to replace inactivated normal cells (Heidenreich et al. 2001).

A strong effect of promotion on the development of lung cancer among the Colorado miners has been reported (section 6.4.1). From these data a quantitative estimate of the promotion of intermediate cells due to a radiation exposure of lungs was derived (Heidenreich et al. 2001). The ratio $\Delta\alpha/\Delta\beta$, where $\Delta\alpha$ is the radiation induced increase of the proliferation rate and $\Delta\beta$ the radiation induced increase of the death or differentiation rate of the intermediate cells, was assessed to be 2.0, if basal cells are the sensitive cells for the induction of lung cancer, and 1.2, if secretory cells are the sensitive cells.

TSCE model and low-dose hypersensitivity. The radiation induced division rate of intermediate cells was assumed by Jacob and Prokić (2002) to be proportional to the cell inactivation of healthy L132 lung epithelial cells

$$\Delta\alpha = c_\alpha (1 - S(D))/\Delta t_p, \qquad \text{(eq. 6.28)}$$

and their inactivation rate to be the same as for the L132 cells

$$\Delta\beta = (1 - S(D))/\Delta t_p, \qquad \text{(eq. 6.29)}$$

where Δt_p is one week, the time span between exposure and measurement of inactivation. Two model variants were studied, one with $c_\alpha = 2.0$ and one with $c_\alpha = 1.2$. In addition, calculations have been performed with the conventional approach to assume that radiation acts only on the initiation rate.

Application to lung cancer incidence among atomic bomb survivors. Whereas there is evidence for the promotional action of protracted radon exposures, it is much less clear whether acute exposures to γ-radiation also cause a promotional effect. Jacob

Tab. 6.4 Parameters of three TSCE models adapted to the lung cancer incidence among male atomic bomb survivors: the spontaneous promotion rate $\gamma(0)$, its change $\Delta\gamma$ for a week due to the promotional effect of the exposure (for two different doses), and the product of the number $c_1\ n_s$ of intermediate cells created per unit dose and the spontaneous transformation rate λ_2 (after Jacob and Prokić 2002).

Radiation acts on:	$\gamma(yr^{-1})$	$\Delta\gamma(yr^{-1})$	$c_1\ n_s\ \lambda_2\ (yr^{-1}\ Sv^{-1})$
Initiation	0.18 0 for 1.0 Sv	0 for 0.1 Sv	4.3×10^{-6}
Initiation and promotion (secretory cells)	0.18	1.2 for 0.1 Sv 4.2 for 1.0 Sv	3.8×10^{-6}
Initiation and promotion (basal cells)	0.18	4.6 for 0.1.0 Sv	2.5×10^{-6}

and Prokić (2002) explored the possible consequences by applying models with promotional effects to the lung cancer incidence data for the atomic bomb survivors. The above discussed model with a radiation action on the promotion of intermediate cells by inactivating basal cells fitted the data slightly better than other models. The fit value of $\gamma(0)$, the spontaneous promotion rate of intermediate cells, is the same in all three models (Tab. 6.4 for males, similar results have been obtained for females). For the period of the promotional effect, the radiation induced promotion exceeds the spontaneous promotion rate considerably, even for doses as low as 100 mGy. The radiation induced initiation of intermediate cells decreases with an increasing effect of the radiation on the promotion.

The excess relative lung cancer risk varies with age at exposure and with sex (Tab. 6.5). According to the constant excess relative risk model, for males the *ERR* was smaller in the age-at-exposure group of 20 to 39 years than in the age-at-exposure group older than 40 years. In contrast, the TSCE model, assuming an exclusive radiation action on initiation, resulted in an risk estimate that is by a factor of 7.5 larger for the younger age-at-exposure group than for the older age-at-exposure group. For the TSCE model, with an assumed radiation action on initiation and promotion, this factor has the value of 2, which is closer to the ratio of the estimates performed with the constant relative risk model. Thus, the implementation of the low-dose hypersensitivity in the TSCE model improved the description of the age-at-exposure dependence of the observed data.

Implications for estimates of excess lifetime risk. In the TSCE model variant with a radiation action on the promotion, the dose dependence of the excess relative risk depends on age at exposure (Fig. 6.16). For low and medium ages at exposure, the dependence of the *ERR* on dose does not differ significantly from linearity. For large ages of exposure, however, the radiation induced promotion of intermediate cells has a large effect because intermediate cells are relatively frequent at higher age. The assumed low-dose hypersensitivity leads to an estimate of the excess risks per unit dose that is larger at low doses than at high doses. At low doses (10 mSv)

Tab. 6.5 Excess relative risk (*ERR*) for lung cancer incidence among the atomic bomb survivors as estimated by a model of constant *ERR* for two age-at-exposure groups and by two versions of the TSCE model for the cohort as a whole (after Jacob and Prokić 2002). Median values and 95 % confidence intervals are given.

Model	Age-at-exposure: 20–39 years		Age-at-exposure: ≥ 40 years	
	Male	Female	Male	Female
Constant *ERR*	0.24 (-0.2; 0.9)	2.0 (1.0; 3.3)	0.7 (0.2; 1.3)	1.9 (0.9; 3.3)
TSCE (radiation acts on initiation only)	0.6 (0.2; 2.0)	2.9 (1.7; 5.2)	0.08 (0.03; 0.3)	0.6 (0.3; 1.1)
TSCE (radiation acts on initiation and promotion[a])	1.2 (0.9; 2.4)	2.8 (1.9; 4.6)	0.5 (0.4; 0.7)	1.0 (0.7; 1.4)

[a] Basal cells are assumed to be the sensitive ones for lung cancer induction.

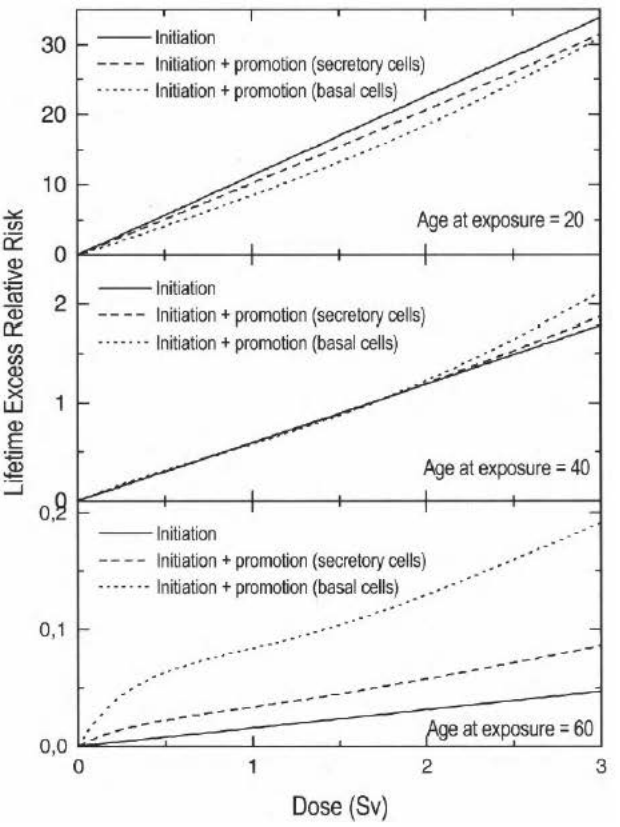

Fig. 6.16 Excess relative lifetime risk for lung cancer incidence among males as derived with three variants of the TSCE model for the cohort of the atomic bomb survivors from Hiroshima and Nagasaki (from Jacob and Prokić 2002). In the first variant radiation acts only on initiation, in the other two variants it acts also on the promotion of intermediate cells, where susceptible cells are either secretory or basal.

the risk per unit dose derived by the TSCE model variant with radiation action on the promotion of intermediate cells (if basal cells are the susceptible cells for lung cancer) happens to be close to the results obtained with the constant relative risk model.

In summary, there is a considerable uncertainty in age, time and dose dependences of organ-specific cancer risk estimates due to a lack of statistical power in radioepidemiological data. The evaluation of these data with a variety of models will elucidate what is known and with which uncertainty. Models of carcinogenesis should be further developed to contain main radiobiological and molecular genetic facts in order to contribute to this task.

6.5 Carcinogenic substances

A main field of modelling the influence of chemical carcinogens on the carcinogenic process is the evaluation of data on the development and size distribution of intermediate lesions. The experimental set-ups allow a sophisticated description of the early processes of carcinogenesis. The application of stochastic models of carcinogenesis on experimental data on the effects of carcinogenic substances has been reviewed by Moolgavkar et al. (1999). Here the state of the art was described exemplarily by reviewing more recent work of modelling of hepatocarcinogenesis in rats, one on the role of cell replication and apoptosis in tumour initiation and one on combined effects of an initiating and a promoting agent. Carcinogenesis in rat liver is of interest for an understanding of carcinogenesis because intermediate stages can be determined experimentally, e. g. by measuring changes of the expression of enzymes (see also section 4.5.1). Placental glutathione *S*-transferase (GST-P) is a useful marker for most (pre)neoplastic lesions (Sato 1989). GST-P positive (G^+) cells appear in liver preneoplasias after administration of various genotoxic hepatocarcinogens, but not after administration of non-genotoxic agents (Moore et al. 1987).

The number of applications of mathematical models of carcinogenesis to epidemiological studies on chemical carcinogens is limited. In the last part of this section, an analysis of lung cancer due to coke oven emissions is summarised.

6.5.1 Replication and apoptosis of intermediate cells in hepatocarcinogenesis

Cell replication and apoptotic activity have been observed in rats to increase from normal liver cells to preneoplasias and neoplasias (Grasl-Kraupp et al. 1997). At all stages of the carcinogenic process, cell replication rates were found to be higher than apoptosis rates.

Recently, first stages of hepatocarcinogenesis were studied by applying *N*-nitrosomorpholine (NNM) to rats (Grasl-Kraupp et al. 2000). NNM is one of the nitrosamines occurring in tobacco smoke and in a variety of foods and alcoholic beverages, and is likely to contribute to the development of human cancer. NNM was administered as a single dose of 250 mg NNM 10 ml^{-1} solution kg^{-1} body weight by gavage to male SPF Wistar rats at the age of 6–8 weeks. Measurements of the cellular dynamics were performed during a period up to 107.5 days after exposure. The exposure caused a high apoptotic effect in the liver. Regeneration started 48 h after NNM application. The total hepatic DNA content and absolute liver weights were back to their original level 24 days after the exposure. Rates of replication and apoptosis were still elevated after 31.5 days.

After NNM treatment, the number of G^+ single cells and small clones per unit area of evaluated tissue sections were scored (Fig. 6.17 A). The number of 3D clones per liver were estimated by a stereological method that took into account the non-spherical shape of the clones (Fig. 6.17 B). Both aspects indicated three phases after NNM application that may reflect alterations in the concentrations of growth factors in the liver. The modelling was performed with the first two stages of the TSCE model. The division rates α and death (or disappearance of G^+ phenotype)

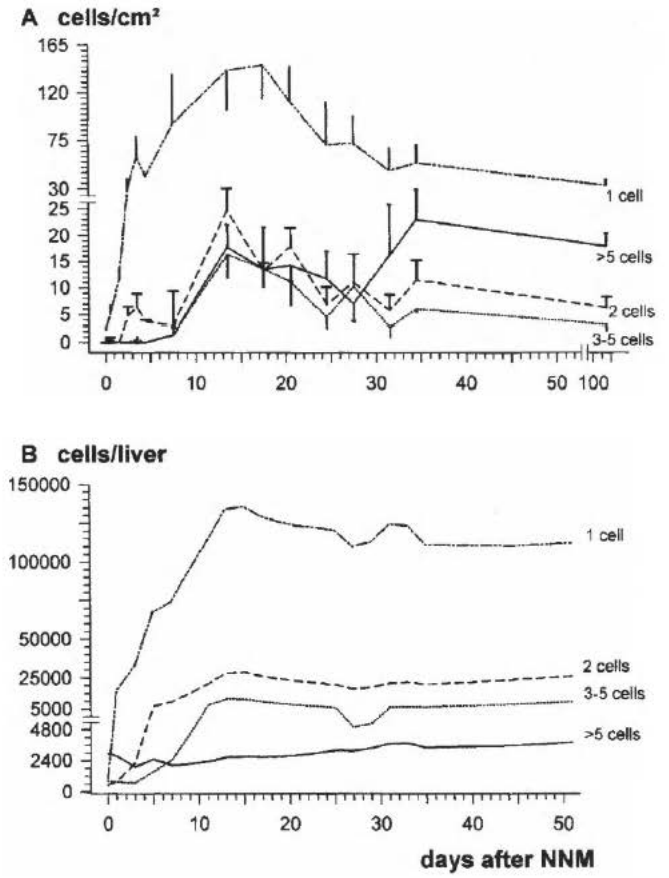

Fig. 6.17 Kinetics of appearance of G^+ single cells and G^+ foci. Experimental data are expressed in cm^{-2} evaluated tissue sections (A), derived results for 3D clones as mean values per liver (B) (from Grasl-Kraupp et al. 2000). Note that the majority of cells that appear as G^+ single cells in the tissue sections belong in the 3D reality to G^+ foci.

rates for G^+ single cells and for G^+ cells in clones with more than 1 cell were allowed to be different.

According to the model fit, about 5,000 G^+ clones were present in the rat liver at the time of NNM administration. Phase I is characterised by a reduction of body weight of the rats with a subsequent recovery. It lasted until day 14 after NNM application and reflects the continuous appearance of G^+ single cells and their development into multicellular foci (Fig. 6.17 B). According to the model, about 170,000 new clones, mainly G^+ single cells appeared. Regeneration signals, released in response to severe damage to the liver, are probably responsible for the growth. Both, measurements and model indicate that the G^+ cell division rate is larger in clones than for single cells (Tab. 6.6).

Phase 2, lasting until 28 days after NNM, was the first two weeks after recovery. The phase is characterised by a loss of G^+ cell clones (about 40,000 after the model

Tab. 6.6 G^+ cell division and disappearance rates in different phases after NNM administration to rats (after Grasl-Kraupp et al. 2000). Experimental results for disappearance rates are apoptotic rates, model results include in addition the loss rate of G^+ phenotype. Mean values (MV) and 95 %-confidence intervals (CI) are given for the model results.

Time after NNM administration (d)	G^+ cell division rates α (% d^{-1})		G^+ cell disappearance rate β (% d^{-1})	
	total (mainly single G^+ cells)	in clones > 1 cell	total (mainly single G^+ cells)	in clones > 1 cell
0–14	2.8	14	–	2
Model: MV and CI	4.2 (3.1; 5.2)	23 (19; 27)	0.0	0 (0; 10)
14–28	1.1	6	–	3
Model: MV and CI	0.5 (0.0; 2.5)	13 (4; 22)	6.2 (4.6; 8.6)	19 (8; 30)
28–107.5	0.02	2.5	–	1
Model: MV and CI	0.5 (0.0; 2.5)	13 (4; 22)	0.2 (0.0; 5.5)	0 (0–90)

fit) and a loss of constituent cells. The division rates of G^+ cells has gone down by a factor of about three, the disappearance rates are significantly increased. Experimentally, the disappearance rate is the apoptotic rate. The model calculations relate to a decrease of the number of G^+ cells, i. e. here no differentiation is made between apoptosis and loss of G^+ phenotype. Both, the division and disappearance rates, are in the clones higher than for single G^+ cells.

In the subsequent phase, the number of G^+ single cells and of multicellular foci appeared to stabilise and an increase in larger foci was observed. According to the model, in this phase about 11,000 new clones appeared until day 51. Compared to phase 2, the gain is mainly due to a significant decrease of the death rates of G^+ cells.

In conclusion, the consecutive development of G^+ single cells into foci with an increasing number of G^+ cells supports the concept that liver preneoplasias are of monoclonal origin and that expression of the G^+ phenotype is heritable by daughter cells. G^+ cells in clones with more than one G^+ cell exhibit an accelerated turnover. Consequently, a large number of clones disappears due to apoptosis or loss of G^+ phenotype, especially during the first weeks after the recovery of the animals from the NNM treatment (as expressed by their body weight).

6.5.2 Combined effects of an initiating and a promoting agent on hepatocarcinogenesis

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is known for its tumour-promoting property in rat liver (Dragan et al. 1992), lung, and skin of hairless mice (see also section 4.5.10). Moolgavkar et al. (1996) and Portier et al. (1996) analysed data on TCDD applications to rats with the TSCE model and found that TCDD has as initiating capacity as well. At the same time, Stinchombe et al. (1995) performed continuative experiments on G^+ cells in the liver of rats after the application of on initiating agent and of TCDD.

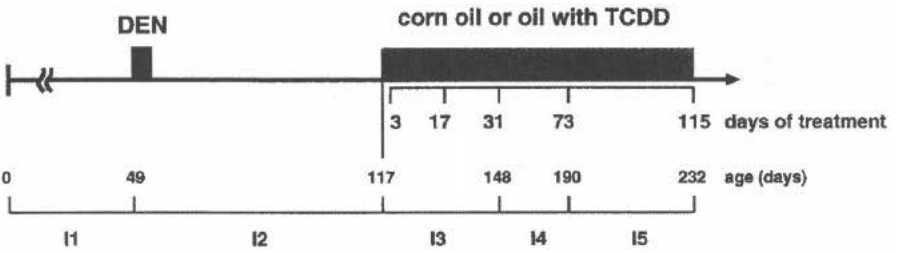

Fig. 6.18 Experimental protocol and intervals (I1,..., I5) for modelling the age dependence of initiation, division and disappearance rates of G^+ liver cells in rats that have been exposed to the initiating agent diethylnitrosamine (DEN) and subsequently to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (from Luebeck et al. 2000).

In the experiments, 7-week-old female Wistar rats were exposed for 10 days to diethylnitrosamine (DEN), an initiating agent, with a dose rate of 10 mg kg^{-1} body weight d^{-1}. After a recovery period of 8 weeks, the animals received biweekly injections with TCDD corresponding to a chronic dose rate of 100 ng kg^{-1} body weight d^{-1} (Fig 6.18). The experiment showed a clear evidence that TCDD suppresses apoptosis in G^+ clones after ten weeks of application. Before this time, G^+ clones are smaller in the TCDD treated animals than in the controls, at later times they are larger.

Luebeck et al. (2000) analysed the data with the TSCE model assuming that the values of the parameters λ_1, α, and β are constant in five time intervals that are defined by the exposure patterns and long term behaviour of the G^+ cells. The number and sizes of G^+ clones in the liver were derived from results on G^+ positive foci in two-dimensional liver transections. The division rate α of G^+ cells was assumed to be independent of age and exposure, as it was indicated by the measurements. A value of 0.06 d^{-1} ml^{-1} of liver was obtained. Three main conclusions can be derived:

- Firstly, the acute treatment with DEN leads to a protracted appearance of initiated G^+ cells, with a massive increase in a period of about 100 to 150 days after the application. This may be related to long-lived DNA adducts that are converted into mutations as cells undergo division.
- Secondly, TCDD treatment appears to accelerate the formation of new clones possibly due to an accelerated conversion of DEN-induced DNA damage into fixed mutations required for an overexpression of the enzyme marker (Fig. 6.19). After the first 30 days of TCDD treatment, the initiation rate does not seem to exceed background any more. In total, less new clones are created than without TCDD exposure, possibly because the cytotoxicity of TCDD removes some of the cells with DEN-induced damage. The accelerating effect of TCDD may have shamed an initiating effect as it was reported in earlier analyses (Moolgavkar et al. 1996; Portier et al. 1996).
- Thirdly, the dynamics of the clonal growth of G^+ cells is complex, however, after longer periods of TCDD treatment (more than about 70 days), TCDD down-regulates the apoptosis of G^+ cells to about 60 % of the control value. This leads to a faster growth of the clones compared to the control implying a larger hazard for the incidence of later stages of the carcinogenic process.

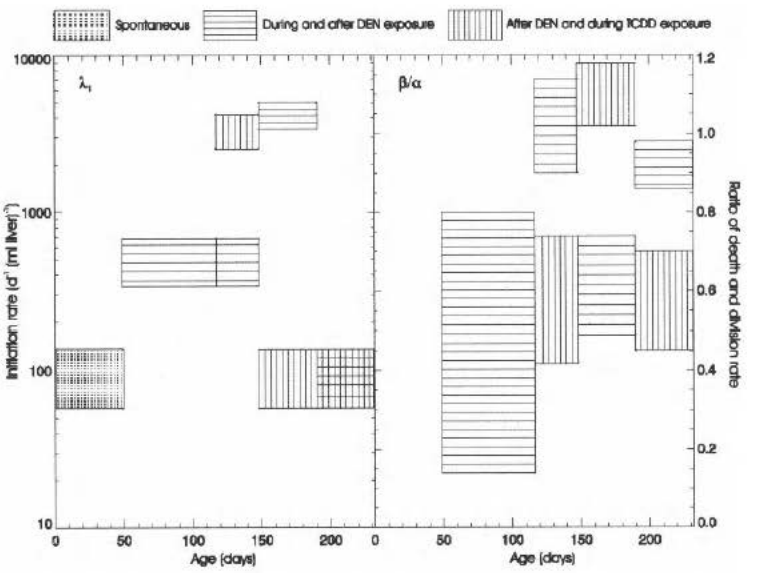

Fig. 6.19 Estimated 95 % confidence ranges of the initiation rate λ_1 and of the ratio β/α of the death and division rates for G^+ cells in clones in female Wistar rats that were exposed to DEN and TCDD (after Luebeck et al. 2000).

6.5.3 Lung cancer due to coke oven emissions

Coke oven emissions contain a complex mixture of polycyclic aromatic hydrocarbons. Moolgavkar et al. (1998) used the TSCE model to analyse the lung cancer mortality among a cohort of coke oven workers and not exposed controls from the same steel plants in the US. All cohort members started to work in the plants in the early 1950s. Detailed work histories were obtained for each worker up to 1982 and also the mortality follow-up extended up to the year 1982. Based on a survey taken in the late 1960s, average concentrations of coal tar pitch volatiles (CTPV) of 3.13 mg m^{-3} were assumed for working places on the top of the ovens and of 0.88 mg m^{-3} on the side of the ovens. Mathematical models of carcinogenesis are particularly suited for analysing such a data set, because complex exposure scenarios can be taken into account without further assumptions or parameters. Information on individual smoking behaviour was not available. The model, however, contained a term for a birth-year dependence in the promotion rate that can be due to a general change of the smoking behaviour within in the cohort.

The cohort was divided in four subcohorts, according to whether the workers were White or non-White, and whether they worked inside or outside of the Allegheny County. For all subcohorts except for Allegheny County Whites, a statistically significant difference was found in the lung cancer mortality among the unexposed and exposed subgroups. The authors concluded that this was possibly a substantial misclassification of exposures for the Allegheny County Whites, because the lung cancer mortality was highest in the second quartile of exposures.

Another possible reason for this pattern might be differences in the individual smoking behaviour about which no information was available. Similarly, for the non-Allegheny County Whites the lung cancer mortality was highest in the third quartile of exposure and there was little indication of an exposure-response relationship.

The main conclusions of the study were based on non-White cohort members who did not work in the Allegheny County. A significant effect of the coke oven emissions on both, the initiation and the promotion rate was found. The preferred model had exponential dependencies of the model parameters on the exposure rate (concentration of CTPV). No effect on the transformation rate was found. The optimal value of the lag time was found to be 3.5 years. Although these finding are remarkably similar to what has been found for uranium miners (see section 6.4.1.2), one should bare in mind that the results could be derived only for one of the four subcohorts.

6.6 Ionising radiation and carcinogenic substances

Although there is quite an extensive literature on effects of combined exposures to ionising radiation and carcinogenic substances (for a review see e. g. Streffer et al. 2000), there is only limited experience on modelling the carcinogenic process after such combined exposures. Work that has been performed on Colorado uranium miners and on Chinese tin miners is summarised in section 6.6.1. Some theoretical work based on TSCE model calculations for combined exposures are outlined in section 6.6.2 in order to demonstrate exemplarily low-dose risk estimates of the model for combined exposures.

6.6.1 Radon, smoking and arsenic

Luebeck et al. (1999) analysed the interaction of radon and smoking in the lung cancer mortality among the Colorado Plateau miners (section 6.4.2). It was assumed that radon and smoking affect the initiation and promotion rates independently. No effect on the transformation rate was found. The increase of the initiation and the promotion rates relative to their spontaneous values consists in both cases of two summands, one for smoking and one for radon exposure (Fig. 6.11). For the exposure scenarios shown in Fig. 6.20, the resulting relative risk is nearly multiplicative in the time period where both exposures apply (plus lag time). This is because smoking and radon are assumed to act additively on the promotion rate, and during the considered age range the hazard increases approximately exponentially with the product of the promotion rate and age (eq. 6.21). At the end of the assumed exposure scenario (plus lag time), the hazard is a bit submultiplicative due to the clones that were initiated by smoking or radon. Subsequently, the hazard becomes considerably smaller than the multiplicative risk and decreases with age (for high ages even below an additive risk model).

Hazelton et al. (2001) used the TSCE model in analysing lung cancer mortality data for a cohort of Yunan (China) tin miners who were exposed to arsenic and radon. The analysis was performed for 12,011 male miners with complete records

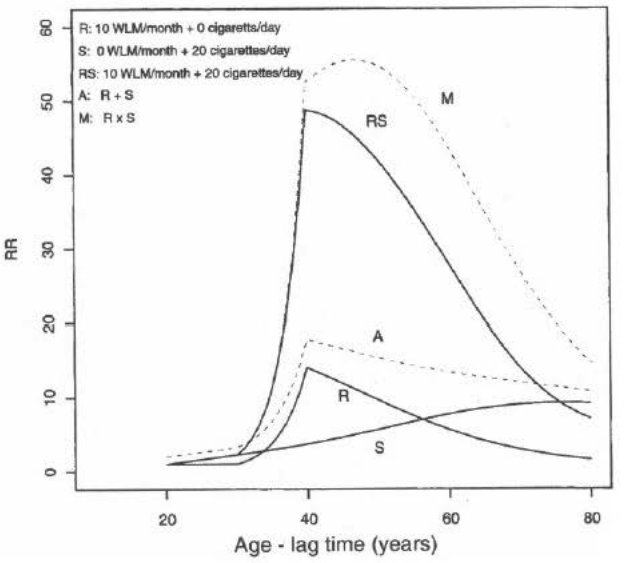

Fig. 6.20 Relative risk of lung cancer mortality at age (a-lag time) according to a TSCE model fit to the Colorado Plateau miners data for single exposures [smoking (S): 20 cigarettes d^{-1} starting age 20; radon (R): 10 WLM $month^{-1}$ between ages 30 and 40], and for combined exposure (RS). Dashed lines (A and M) show strictly additive and multiplicative models, respectively (from Luebeck et al. 1999).

of arsenic, radon, and cigarette and pipe smoke exposures. In the follow-up period 1976 to 1987, 842 lung cancer deaths were reported that amounted to 48 % of all deaths. Additional risk factors like workplace exposures to silica or other carcinogens, residential exposure to smoke from poorly ventilated indoor cooking and heating, or others may have contributed to this high lung cancer mortality.

In the modeling, the complex information on the different exposure patterns was translated in piecewise constant parameters for several periods from birth to censoring date (end of follow-up, date of death or loss to follow-up). The authors optimised exposure rate dependencies of the parameters by performing calculations for a large number of possible models. Allowance was made for a birth year effect on the initiation rate. Finally they eliminated all parameters that contributed insignificantly to the likelihood, and allowed the lag time to vary according to a γ-distribution. This "final model" contained 14 free parameters.

Although tobacco smoke has been taken into account, a strong birth year dependence of the initiation rate was found. The analysis indicates that exposure to arsenic, radon and tobacco smoke increases division, death and transformation rates α, β, and λ_2 of intermediate cells. The fitted lag-time distribution has a mean value of 4.1 years with a variance of 2.9 years. The results are not compatible with spontaneous transformation rates $\lambda_2(0)$ exceeding a value of 8.6 10^{-12} yr^{-1}. This suggests that at least two sequential mutations are required after initiation for malignant con-

version. However, taking into account the large number of fitting parameters, one should be careful in generalising these results.

6.6.2
Low-dose risk after exposure to an initiating and a promoting agent

As demonstrated by the example in section 6.5.2, interactions of initiating and promoting carcinogens may be very complex. However, it is illustrative to study the effects of combined exposures to initiating and promoting agents with a model under the assumption that the effect of the promoting agent depends only on the number and sizes of the clones of initiated cells, and not on other biochemical changes that were induced by the initiating agent (Jacob and Prokić 2003).

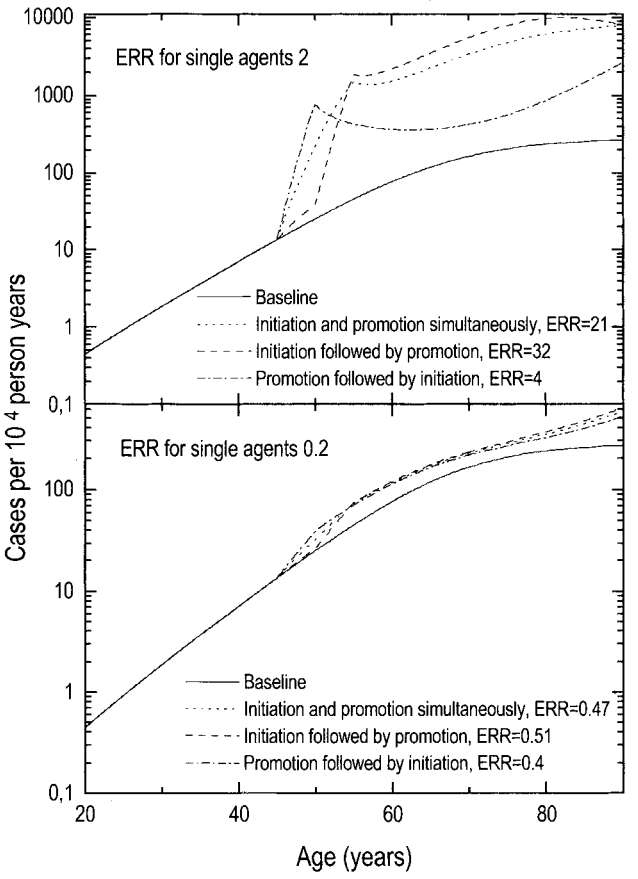

Fig. 6.21 Hazards for solid tumours as calculated with the TSCE model for simultaneous and consecutive exposures to initiating and promoting agents in the age period of 40 to 50 years. The exposures to the single agents result in an excess relative lifetime risk of 2 and 0.2, respectively (Jacob and Prokić 2003).

The TSCE model hazard due to single exposure during the age period of 40 to 50 years has been discussed in section 6.3.1.2. For a combination of the exposures to the initiating and promoting agent, there is a strong synergistic effect for high exposures (ERR = 2 for the single agents), if the agents act simultaneously or if the exposure to the promoting agent follows exposure to the initiating agent (Fig. 6.21). In the first case the synergistic effect amounts to a factor of 5, in the second case to a factor of 8. The reason for the strong synergistic effect is that in these two cases the promotion acts on the clones that are created by the initiating agent. This is not the case, if the exposure to the initiating agent follows the exposure to the promoting agent. The effects on the hazard are additive.

For low exposures (ERR = 0.2) the synergistic effect becomes negligible. Compared to the high exposure it decreases from a factor of 5 to 1.2, resp. from a factor of 8 to 1.3. The reason is that for low exposures the change of the distribution of spontaneously created clones of intermediate cells due to the initiating agent is minor. Therefore, even if the promoting agent can act on the clones created by the initiating agent, the corresponding contribution is small compared to the effect of the promoting agent on the spontaneously created intermediate cells. Having in mind, that an exposure leading to an excess relative risk of 0.2 is in general large compared to exposures resulting from environmental contaminations, the synergistic effect is negligible for environmental contaminations with initiating and promoting agents, if the effect of the promoting agent depends only on the number and sizes of the clones of initiated cells, and not on other biochemical changes that were induced by the initiating agent. These features of the TSCE model are in full accordance with what has been observed experimentally and with other mechanistic models (see Streffer et al. 2000).

6.7 Conclusions

Mathematical models of carcinogenesis have been applied to data of radioepidemiological studies and of animal experiments with exposures to ionising radiation and to carcinogenic substances. Compared to heuristic epidemiological models, as the excess relative risk model, mathematical models of carcinogenesis have in general a smaller number of free parameters, fit the data equally well, and have the advantage of a straightforward treatment of complex exposure scenarios.

The large potential of mathematical models of carcinogenesis in the application to epidemiological data is in the assessment of risks in the low-dose range by taking into account epidemiological, radiobiological and molecular genetic data. Further, the models relate numbers and sizes of intermediate stages as adenomas or liver foci to cancer incidence or mortality rates in the same population.

In the application to an evaluation of experimental studies the potential of mathematical models of carcinogenesis lies in an improvement of the understanding of the dynamics of early stages of the process and in the generation of hypotheses that can be tested experimentally. This has been proven, e. g. for studies on the behaviour of placental glutathione *S*-transferase positive (G^+) cells which have the capacity to form preneoplasias in rat liver.

Comparative studies with lung cancer data for rats that were exposed to radon progeny and with cancer incidence data for the atomic bomb survivors have shown

that the statistical power of these data sets is not large enough to decide which is the most appropriate model. Simplicity, plausibility, and radiobiological and molecular genetic knowledge are additional aspects to identify appropriate models. According to the current state of art, a variety of models is possible.

The two-step clonal expansion (TSCE) model has been studied quite intensively. It has been shown to predict direct and inverse dose rate effects, depending on the exposure scenario and on the exposure rate dependence of the initiation and promotion rates of intermediate cells. At the same time, in most applications to epidemiological data, parameter estimates had a large uncertainty. Therefore, conclusions on age, time and exposure dependences for single cancer types remain to be uncertain. The application of a variety of models (including phenomenological epidemiological models) offers, however, the possibility to identify common predications and their uncertainties including the model uncertainty.

Interactions of initiating and promoting carcinogens have been studied with the TSCE model. As to be expected, the degree of synergistic effects depends on the exposure scenarios, e. g. which carcinogen is applied first. However, according to the model, synergistic effects tend to become negligibly small if the doses of both carcinogens are small.

In the past one or two decades, several low-dose radiobiological effects have been discovered. The biological response to low doses of ionising radiation may be quite different from that at medium and high doses. These include low-dose hypersensitivity, bystander effects, gene expressions and genomic instability (see chapter 3). Models of carcinogenesis offer the possibility to integrate such effects, which is not so easily done with phenomenological models. A first example of such work is the integration of low-dose hypersensitivity concerning cell inactivation in the TSCE model and its application to lung cancer incidence among the atomic bombs survivors. The example demonstrates possible consequences for low-dose risk estimates. More work in this direction is to be expected in the future.

For a large class of models, the stochastic multistage models with clonal expansion, the exploration of their features is still in an early stage. A main weakness of the currently existing approaches in this field is the vast number of possible processes and free parameters. The growing experimental experience on processes of carcinogenesis and genetic pathways, however, may offer the possibility of a fruitful application of such models in the future. A first impressing step has been made with applying a four-step model to population data on colorectal cancer.

6.8 References

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6.9 Appendix

6.9.1 Likelihood, model fits and deviance

In general, in a cohort study for each cohort member i, the age of entry a_{1i} and the censoring age a_{2i} are known. In a model that describes the probability $\Psi_i(a\text{-}t)$ that for the exposure history i none of the susceptible cells has become malignant at age a-t, the probability or likelihood that the cohort member did not receive a cancer in the period (a_1, a_2) is

$$L_{\text{nocase},i} = \Psi_i(a_{2i}\text{-}t) / \Psi_i(a_{1i} - t). \qquad \text{(eq. A.1)}$$

The likelihood, that he died of a cancer (mortality data) or received a cancer (incidence data) is

$$L_{\text{case},i} = \dot{\Psi}_i(a_{2i} - t) / \Psi(a_{1i} - t). \qquad \text{(eq. A.2)}$$

In order to fit a model to the data, the product L of the likelihoods $L_{\text{nocase},i}$ for those who did not die because of (receive a) cancer and $L_{\text{case},i}$ for those who died because of (received a) cancer is maximised by varying the model parameters. This is called an individual likelihood technique.

In some studies, e. g. studies the atomic bomb survivors with publicly available data, instead of individual data, only the numbers of cancer cases N_j in subcohorts (strata) j are known. The expectation value E_j for the number of cases in a stratum is calculated by multiplying the person-years of observation in the stratum with the model hazard $H_j(a_j)$ for the age, time and exposure values for the stratum. The likelihood to have the number of observed cases N_j in the given stratum is calculated with a Poisson distribution with the expectation value E_j. The product L of the likelihoods over all strata j is then maximised to varying the model parameters. This is referred to a Poisson regression technique.

For most cases discussed here, there are no rigorous procedures to compare the quality of fit of different models. However, the deviance, which is twice the difference of the natural logarithm of their likelihoods, is often used as an approximate measure for the fit quality. In a family of models, compared to a model with a small number of parameters, a model with one additional parameter is considered to be an improvement on the 95 % confidence level, if its deviance is smaller by a value of 4, a model with two additional parameters, if its deviance is smaller by a value of 6, and a model with three additional parameters, if its deviance is smaller by a value of 8 (James 1994).