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Hematotoxic effects of benzene analyzed by mathematical modeling

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Summary

The hematopoietic cell response to benzene intoxication in mice (during and after long-term inhalation) was analyzed by a mathematical model of murine hematopoiesis. Two complementary methods, Time-Curve and Steady-State Analysis, were developed to identify target cells for benzene toxicity and to quantify the extent of damage in different stages of development of these target cells. We found that (i) erythropoietic cells were the most sensitive; (ii) granulopoietic cells were about half as sensitive as erythropoietic and (iii) hematopoietic stem cells exhibited a sensitivity that ranged between that of erythropoietic and granulopoietic cells. A dose-response relationship between benzene levels and damage in target cells (valid from 1 to more than 900 ppm) was derived that was linear for doses up to 300 ppm and plateaued thereafter. This relationship indicated that benzene-induced hematotoxicity is subject to a saturable process. Recovery of hematopoiesis following chronic benzene intoxication was simulated for different doses and preceding exposure periods. The impaired recovery following exposure periods >8 weeks could be explained by a severe reduction in the maximum self-maintenance of stem cells. This study indicates that the present mathematical model represents a useful approach to investigate alternate hypotheses for the action of hematotoxic agents.

Key words: Benzene; Mathematical model; Toxicity analysis; Hematopoiesis

Introduction

Benzene is one of the most widely used industrial chemicals. It is hematotoxic and carcinogenic to both humans and animals [5,7,14,41]. Extensive experimental studies have been carried out detailing its hematotoxic effects as well as its metabolism and pharmacokinetics (for reviews see Refs. 9,16,21,41).

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Chronic benzene inhalation (≥ 100 ppm) is reported to cause a severe reduction of hematopoietic stem and erythropoietic cells as well as granulopoietic progenitors [6–8,32,33]. In contrast, reports on differentiated granulopoietic marrow and blood cells are ambiguous [4,6,11,12,28,37–40].

Consideration of only the experimental data makes it difficult to decide to what degree the observed changes in the different hematopoietic cell stages result from direct toxic effects of benzene or indirect effects via compensatory regulatory mechanisms. Our objective is to demonstrate how this dilemma can be approached by a suitable mathematical model of murine hematopoiesis that permits the quantification of damage in different cell stages.

Experiments reported previously have been carried out over a broad range of doses (1 to more than 4000 ppm) showing that different regimes with the same cumulative dose (e.g., 316 ppm for 19 day vs. 3000 ppm for 2 days), exhibit quite different hematotoxic effects [7,11]. Moreover, pharmacokinetic studies indicate that benzene uptake and metabolism are saturated for doses greater than 400–600 ppm [30]. This prompted us to derive a dose-response relationship in order to (i) simulate the experimental data over a broad range of doses, (ii) explain the observed differences with respect to exposure regimen and (iii) test whether the phenomenon of saturation exists for benzene-induced hematotoxicity.

The recovery of hematopoiesis following termination of chronic benzene intoxication has been studied for several doses and exposure periods. [6–8,32–34,38]. These data indicate that hematopoietic stem cell recovery is rapid and complete for exposure periods less than 8 weeks (e.g., recovery within 2 weeks after inhalation of 300 ppm for 2 and 4 weeks). Longer exposure periods result in a considerable delay in stem cell recovery, indicating a benzene-induced residual hematopoietic injury. For example, following a 16-week exposure to 300 ppm, stem cells do not return to normal values for an additional 16 weeks [6,7].

We investigated the impaired recovery of hematopoiesis with the model in order to identify and quantify the specific benzene-induced hematopoietic injury in terms of distinct model parameters.

Methods

Standard model and definitions

The mathematical model used (schematically summarized in Fig. 1) is a combination of a stem cell and mature erythropoiesis model [22,45]. Briefly, the mathematical description is based on compartments each comprising a single morphologically defined stage of cell differentiation. They are characterized by a transit time T, a cell cycling activity a and either an amplification coefficient Z (in non-self-renewing compartments) or a self-renewal probability p. Changes in a compartment size Y with time t are described by ordinary differential equations of the type:

$$dY/dt = C^{in} * Z - a * Y/T \quad (1)$$

with C^{in} representing the cell input rate. The amplification coefficient Z appearing

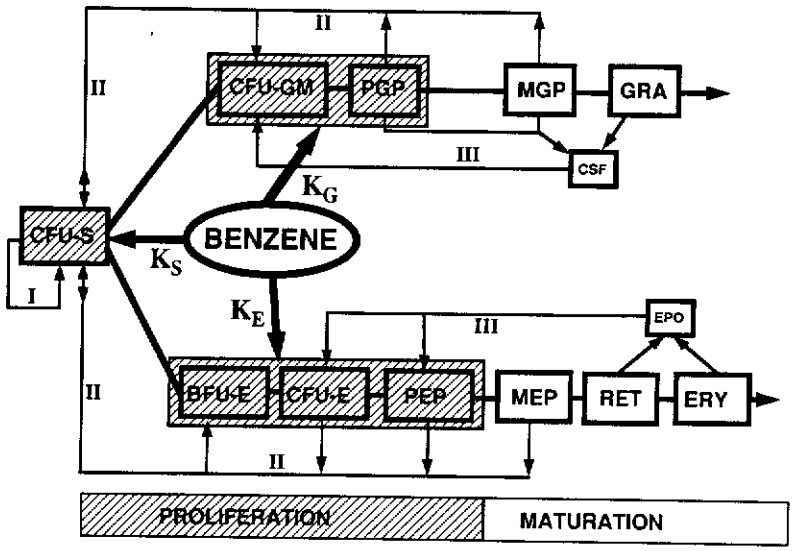

Fig. 1. Structure of the model: Granulopoiesis and erythropoiesis descend from stem cells (CFU-S). Thrombopoiesis is not considered. Hematopoiesis is regulated by three types of feedbacks (thin arrows). Feedback I: autoregulation of CFU-S. Feedback II: intramedullary feedback from erythro- and granulopoietic bone marrow cells to CFU-S, BFU-E and CFU-GM. Feedback III: feedback from reticulocytes (RET) and erythrocytes (ERY) to CFU-E and proliferating erythroid precursor cells (PEP); feedback from proliferating (PGP) and maturing (MGP) granulopoietic precursors and granulocytes (GRA) to CFU-GM. Benzene is assumed to act on all proliferating cell stages with the loss coefficients k_S, k_E and k_G quantifying the damage in CFU-S (k_S) erythroid (k_E) and granuloid cells (k_G) The model hormones for erythropoiesis and granulopoiesis are denoted by EPO (erythropoietin) and CSF (colony-stimulating factor(s)), respectively.

in the input term with the output being proportional to the compartment content (first order kinetics), makes simple numerical calculations possible.

To calculate hematotoxic effects on granulopoietic cells, an additional series of compartments for granulocytes has been added with humoral feedback from bone-marrow and blood granulocytes on granulocyte-macrophage progenitor cells. The granulopoietic precursor compartment is divided into proliferating and non-proliferating precursors. The model parameters either are taken directly from the literature or fixed in the course of simulating various experiments (described in Refs. 22,45,46).

Control processes

The regulation of hematopoiesis is governed by three interrelated feedback loops: autoregulation of stem cells (feedback I), intramedullary feedback (feedback II) and feedback from mature cells to progenitors and precursors (feedback III).

Stem cell (colony forming units, spleen or CFU-S) regulation

Two distinct properties of CFU-S are regulated via feedback I and II; namely their cyclic activity and their potential for self-renewal. The first parameter is defined as the fraction of CFU-S in active cell cycle (a_s). It determines the turnover rate of

stem cells and thus the rate of cell production. Self-renewal is defined as the property of CFU-S to maintain, after cell division, the same characteristics as the cell of origin. It is quantified by the self-renewal probability p_s. A reduction in CFU-S numbers increases self-renewal and cycling (feedback I), a lack of differentiated cells stimulates stem cell cycling, but induces a decrease in p_s (feedback II).

Regulation of committed erythropoietic and granulopoietic cells

The model assumptions on erythropoietic and granulopoietic regulation have been described in detail elsewhere [22,35]. Briefly, three properties of committed cells are regulated: (i) Variable cell cycling activities are assumed for the early erythropoietic (burst-forming unit, erythroid or BFU-E) and granulopoietic progenitors (colony-forming unit, granulocyte-macrophage or CFU-GM). Decreases in CFU-S and/or progenitors and precursors of both cell lines increase the cycling activities $a_{\text{BFU-E}}$ and $a_{\text{CFU-GM}}$ (feedback II). (ii) The proliferating erythropoietic precursor's transit time (T_{PEP}) depends on erythropoietin (EPO) such that high levels of EPO induce a shortening of the transit time. (iii) Amplification in the late erythropoietic progenitors (colony-forming unit, erythroid or CFU-E) and proliferating erythropoietic precursors (PEP) is controlled by EPO and in CFU-GM by CSF (colony-stimulating factor(s)) (feedback III). Amplification is defined as the increase in cell number by cell division that can be quantified by the amplification coefficient Z, denoting the number of descendants produced per progeny (Z = cell output/cell input).

Simulation of benzene toxicity

To model the continuous effect of benzene intoxication, we assume that: (i) Benzene acts on early proliferating cells (i.e., CFU-S, progenitors and proliferating precursors). This is consistent with experimental results demonstrating that benzene affects replication and transcription [16–18,29] and results in covalent binding of metabolites to DNA [9,16,23,42]. (ii) Loss coefficients quantify the destruction rates (= relative loss of cells in the affected compartments). They are constant with time. (iii) Proliferating cells of one cell lineage are affected to the same degree. Thus, three different loss coefficients k_S, k_E and k_G quantify the damage in CFU-S (k_S) proliferating erythropoietic (k_E) and granulopoietic (k_G) progenitor and precursor cells (Fig. 1). Mathematically, this leads to the following equations:

$$dY_s/dt = (2p_s - 1)\ Y_s * a_s/T_S - k_S * Y_S \tag{2}$$

for CFU-S and

$$dY_i/dt = C_i^{in} * Z_i - a_i * Y_i/T_i - k_j * Y_i \tag{3}$$

with i = BFU-E, CFU-E, PEP, CFU-GM, PGP (proliferating granulopoietic precursors); j = E, G for proliferating erythropoietic or granulopoietic cells.

Identification of benzene target cells (toxicity analysis)

Time-Curve Analysis. Time-curves are created by running the model simulations for different combinations ('Toxicity Patterns') of k_S, k_E and k_G. A 'Toxicity Pattern' is defined by a fixed ratio of loss coefficients independent of the simulated dose (i.e., k_S/k_E, k_E/k_G and k_S/k_G are identical for all doses). Coefficients are limited to three different values ('strong', 'moderate' and 'no' damage) for each dose. The time-curves are compared directly with the experimental data with respect to time course and extent of changes. Those model curves that diverge from at least one dose data set (e.g., increase in model curve while experimental data decrease) are not considered representative of the benzene-specific toxicity pattern. All other model curves/toxicity patterns are considered representative.

Steady-State Analysis. Steady-State curves are created as follows: First, model time-curves are generated for the entire range of doses for each toxicity pattern. After a 60-day simulation of benzene inhalation, cell numbers of the different compartments reach a new plateau with the height of this plateau dependant on the toxicity pattern as well as the simulated dose. Next, these (plateau-) day-60 cell numbers that have been calculated using the same toxicity pattern are plotted against each other for two different compartments. The experimental data are plotted correspondingly. The toxicity pattern of benzene is identified by comparison of the model curves with the experimental data. As only a few experimental studies exist in which the exposure period is long enough to establish a steady-state, we decided to make use of all data that were obtained after an exposure period of at least 28 days.

Dose response relationship

The following mathematical equation was chosen to describe the relation between a given dose D and a loss coefficient k:

$$k(D) = A - A * e(^{-B * D^E}) \tag{4}$$

with $A = k^{max}$, D = dose [ppm * 10^{-3}]. The constants B and E were chosen in such a way that (i) the loss coefficients for 100, 300 and 400 ppm simulated the experimental data satisfactorily and (ii) the curve approaches its maximum (k^{max}) at a range between 400 and 600 ppm.

Simulation of recovery from chronic benzene intoxication

Since the preceding exposure phase is not considered explicitly, the median steady-state values measured during the exposure period are taken as starting values for the model calculations. Four experiments are considered at a dose of 300 ppm given 6 h/day, 5 days/week for: (i) 2 weeks; (ii) 4 weeks; (iii) 8 weeks and (iv) 16 weeks. The corresponding initial model values are: (i,ii): CFU-S, BFU-E, CFU-E, CFU-GM = 0.7; precursors and blood cells = 0.8 and (iii,iv): CFU-S, BFU-E, CFU-E, CFU-GM = 0.4; precursors and blood cells = 0.7. The speed of CFU-S recovery is determined by the output of CFU-S:

$$S^{out} = 2(p_S - 1)\, a_S/T_S * Y_S \tag{5}$$

(for exact derivation of the equation see Ref. 45). The effects of varying the a_S:T_S ratio and the maximum self-renewal probability p_S^{max} on CFU-S recovery time were investigated.

Results

Time-Curve Analysis

It has been reported that genetic differences at the Ah-locus influence the inducibility of metabolizing enzymes and the hematotoxicity of benzene

TABLE I

ABILITY OF DIFFERENT MODEL SIMULATIONS TO REPRODUCE THE EXPERIMENTAL DATA FOR EXPOSURE TO 100, 300, AND 400 PPM OF BENZENE. '⊖' INDICATES CONSIDERABLE DIFFERENCE BETWEEN EXPERIMENTAL DATA AND MODEL CURVES CALCULATED ON THE BASIS OF A GIVEN TOXICITY PATTERN. '⊕' INDICATES THAT THE MODEL CURVES ARE CONSISTENT WITH THE DATA. THOSE MODEL CURVES THAT DIVERGE FROM AT LEAST ONE DOSE DATA SET (I.E., WITH AT LEAST ONE '⊖') ARE NOT REPRESENTATIVE OF THE BENZENE-SPECIFIC TOXICITY PATTERN, WHILE THOSE MODEL CURVES/TOXICITY PATTERNS WHICH ARE CONSISTENT WITH ALL THREE DATA SETS (I.E., 3 × ⊕, SHADED AREA) ARE CONSIDERED REPRESENTATIVE.

		Damage[1] in Proliferating Granulopoietic Cells			
		strong	moderate	no	dose[2]
Damage[1] in CFU-S	strong	⊖	⊕	⊕	100
		⊕ Fig 2a-c Curve 1	⊕	⊖ Fig2a-c Curve 3	300
		⊕ Fig 2d-f Curve 1	⊕ Fig 2d-f Curve 2	⊖	400
	moderate	⊖	⊕	⊕	100
		⊖	⊕	⊖ Fig 2a-c Curve 5	300
		⊕	⊕ Fig 2d-f Curve 4	⊖	400
	no	⊖	⊖	⊖	100
		⊖	⊖	⊖ Fig 2a-c Curve 6	300
		⊕	⊖	⊖ Fig 2 d-f Curve 6	400

[1]Strong damage: 0.47, 1.3 and 1.5% loss per hour (i.e., k_S, k_G = 0.0047, 0.013 and 0.015 h^{-1}) for doses of 100, 300 and 400 ppm benzene respectively; moderate damage: 0.234, 0.65, 0.75% loss per hour for doses of 100, 300 and 400 ppm benzene, respectively; no damage: 0% loss per h. A strong damage to erythropoietic cells is assumed for all calculations.

[2]Benzene exposure (in ppm).

[24,25,27,39,43]. Therefore, the analysis is restricted to those experiments that were conducted on mice with genotype $Ah^{b/b}$ or $Ah^{b/d}$ (so-called 'responders', [1]). Previously published experimental results showing a considerable reduction of all erythropoietic cell stages in mice exposed to 100, 300, or 400 ppm [3,4,7,8,32,33] could be reproduced by assuming strong damage to proliferating erythropoietic cells as defined by a loss of 0.47%, 1.3% and 1.5% loss per hour (i.e., k_E = 0.47, 1.3 and 1.5 × 10^{-2} h^{-1}) in case of 100, 300 and 400 ppm, respectively. Therefore, simulations were carried out assuming these k_E values while the loss coefficients for CFU-S and proliferating granulopoietic cells were varied (i.e., nine different toxicity patterns).

Table I summarizes how well the model calculations based on different model toxicity patterns fit the experimental data. Those model toxicity patterns assuming either a strong or no effect on proliferating granulopoietic cells and/or no effect on CFU-S result in considerable differences for at least one dose. Only two combinations of loss coefficients (representing strong damage to proliferating erythropoietic cells, moderate damage to proliferating granulopoietic cells and either moderate or strong damage to CFU-S) lead to an adequate fit to each of the three doses. Accor-

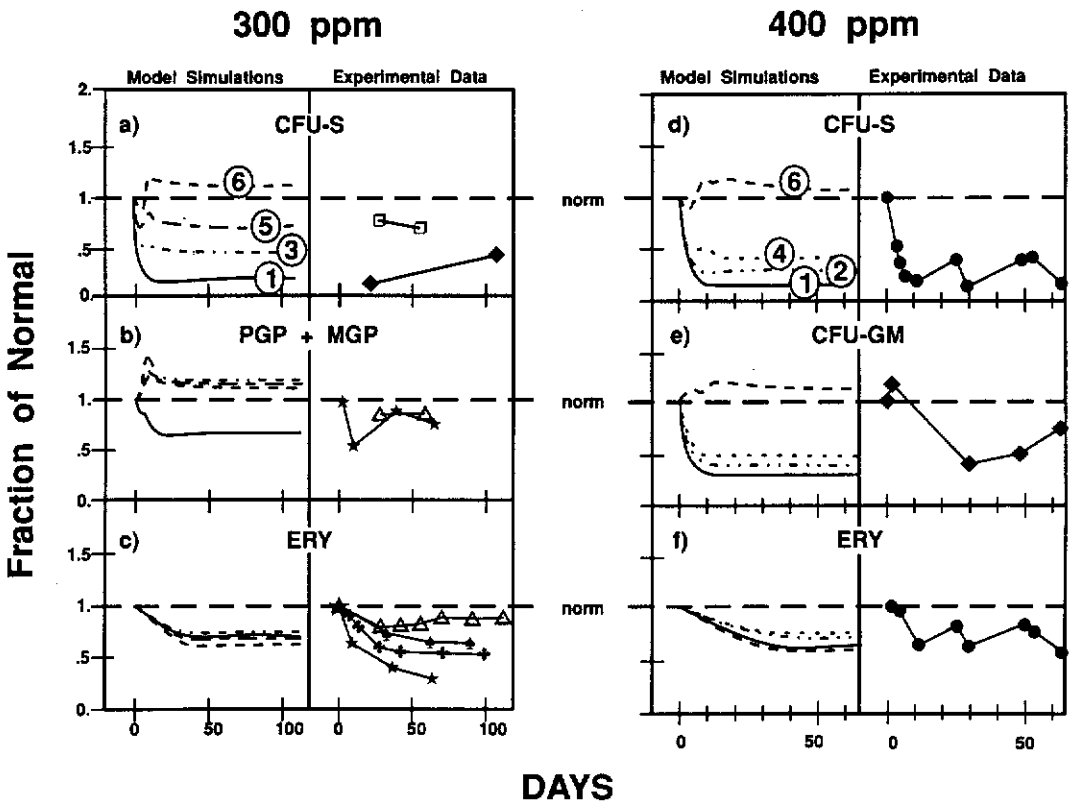

Fig. 2. Comparison of Time-Curve Analysis model simulations (curves 1–6) with experimental data (symbols) for benzene exposures of 300 and 400 ppm. For the relationship of the numbered model curves to those toxicity patterns forming the basis of the simulations, see Table I. Data: ♠, Baarson et al. [3]; ◆, Cronkite et al. [7]; ●, Cronkite et al. [8]; ★, Rosenthal et al. [28]; □, Seidel et al. [32]; △, Seidel et al. (unpublished data) and +, Snyder et al. [36].

ding to this analysis, erythropoietic cells are most sensitive to benzene; granulopoietic cells are only about as half as sensitive as erythropoietic cells with the sensitivity of CFU-S ranging between that of erythro- and granulopoietic cells.

Six (out of nine) model toxicity patterns and their corresponding model time-curves for doses of 300 and 400 ppm are shown in Fig. 2 to illustrate the fit of these curves with the experimental data. Model curves 2 and 4 which adequately reproduce the data for 100, 300 and 400 ppm (for 100 and 300 not shown) were calculated assuming that proliferating granulopoietic cells were moderately damaged, whereas CFU-S were strongly (curve 2) or moderately (curve 4) affected. All curves fit the data for erythrocytes well (Fig. 2c,f). However, curves 3, 5 and 6 exhibit considerable differences with respect to CFU-S and granulopoietic data. Curves 3 and 5, which assume a strong or moderate damage to CFU-S and no damage to proliferating granulopoietic cells, predict an increase above normal in granulopoietic precursors (PGP + MGP), whereas these cells were, in fact, reduced by 300 ppm (Fig. 2b). Curve 6 (no damage to CFU-S and proliferating granulopoietic cells) predicts an increase in CFU-S and CFU-GM rather than the experimental decrease measured (Fig. 2a,d,e). Curve 1 (severe damage to both CFU-S and proliferating granulopoietic cells) more or less fits the data for 300 and 400 ppm benzene. However, it poorly fits the data for 100 ppm. Whereas the model curves for granulopoietic progenitors and precursors show a marked decrease, experimental data do not significantly differ from control values (not shown).

Steady-State Analysis

The model steady-state curves for erythropoietic (PEP + MEP) versus granulopoietic (PGP + MGP) precursors are shown in Fig. 3. Each of these curves correspond to a distinct toxicity pattern (numbering of curves identical to Fig. 2).

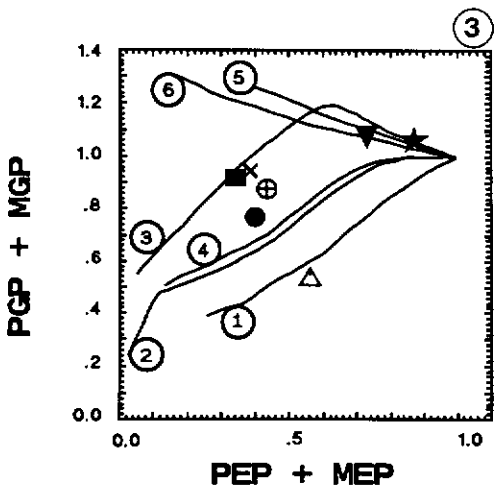

Fig. 3. Comparison of steady-state model curves (curves 1–6; numbering corresponding to Table I) for erythropoietic (PEP + MEP) and granulopoietic precursors (PGP+ MGP) with experimental steady-state cell numbers (symbols). Data: △, 300 ppm, day 32; ⊕, 300 ppm, day 63; ×, 300 ppm, day 88, Baarson et al. [4]; ■, 300 ppm, day 35; ●, 300 ppm, day 63, Rosenthal et al. [28]; ★, 900 ppm, day 28 and ▼, 900 ppm, day 56, Seidel et al. (unpublished data).

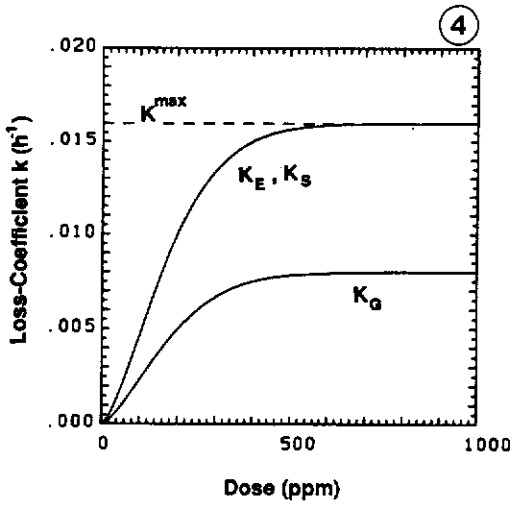

Fig. 4. Dose response curve relating benzene exposure levels to damage in CFU-S (k_S) erythropoietic (k_E) and granulopoietic cells (k_G). The curve is linear for doses up to 300 ppm and shows saturation at doses above this level.

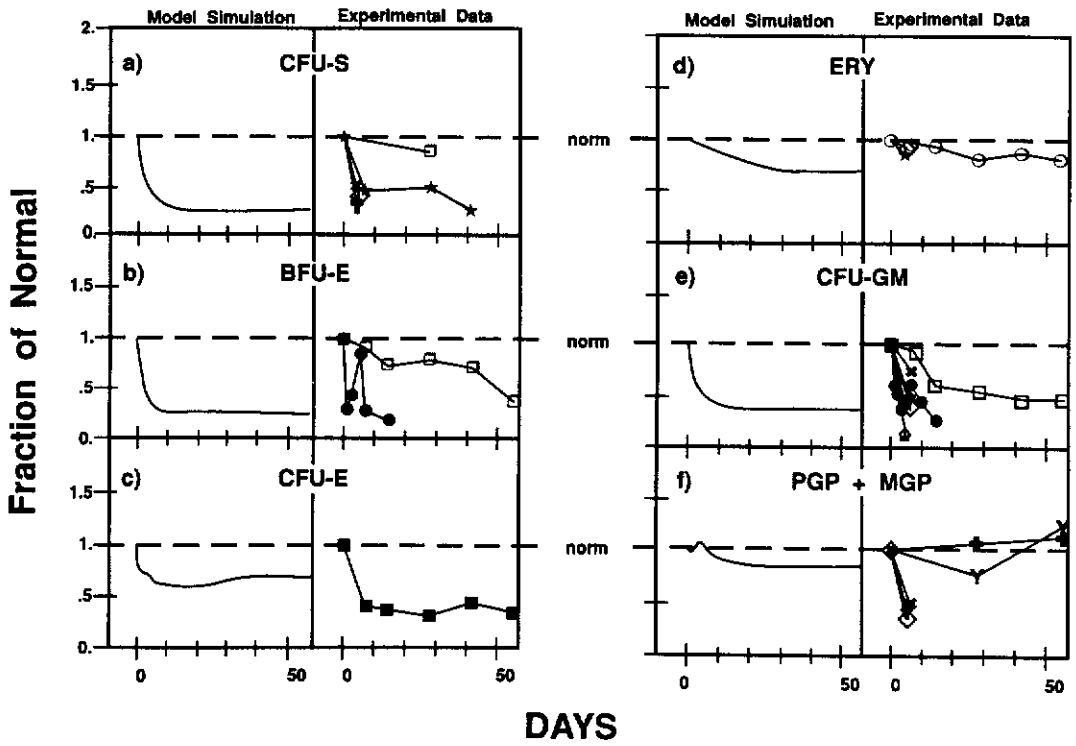

Fig. 5. Comparison between model curves (solid line, toxicity pattern: $k_E = k_S$ = strong, k_G = moderate) and experimental data (symbols) for doses ≥900 ppm. Data: □, 900 ppm, Seidel et al. [32]; Y, 900 ppm, PGP; +, 900 ppm, MGP, Seidel et al. (unpublished data); ○, 900 ppm, Seidel et al. [33]; ★, 4000 ppm, Gill et al. [10]; ×, 1276 ppm; ◇, 2416 ppm; 1, 4862 ppm, Green et al. [11,12]; ●, 4000 ppm, Hilderbrand et al. [15] and ⛢, 4680 ppm, Uyeki et al. [44].

The experimental (steady-state) values derived from the literature are plotted in the same manner. Model curves 2, 3 and 4 encompass the range of all but one of the experimental data points, thereby identifying these curves as potentially representing the benzene-specific toxicity patterns.

Dose response

The relation between a given dose of benzene and the corresponding loss coefficient (here: $k_S = k_E$ and $k_G = 0.5 * k_E$) used in the model is given in Fig. 4. The dose response curve is linear for doses up to 300 ppm and approaches its maximum (k^{max}) at higher doses. This implies a saturation of benzene-induced hematotoxicity.

Effect of saturation

To illustrate this effect, the model time-curves for a dose of 900 ppm and the experimental data for doses $\geq$900 ppm are contrasted in Fig. 5. The simulation

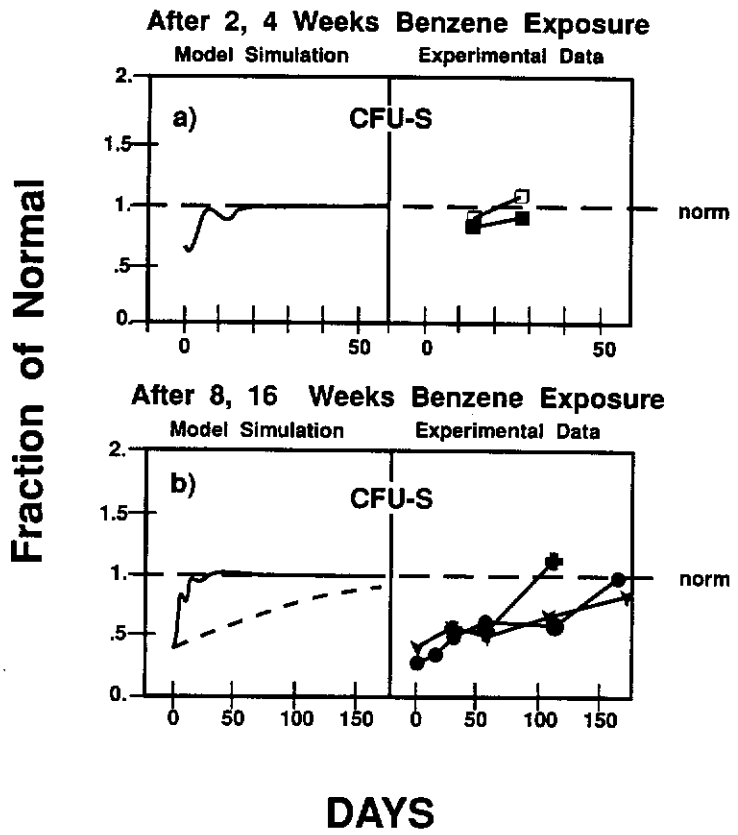

Fig. 6. Comparison of model curves to experimental data for recovery of CFU-S from an inhalation of 300 ppm for either 2 or 4 weeks (Fig. 6a) or 8 or 16 weeks (Fig. 6b). Calculations were carried out without any modification of the basic model assumptions (Fig. 6a,b; solid line) or assuming a considerable reduction in the self-renewal probability of CFU-S to $p_s^{max} = 0.503$ (norm: $p_s^{max} = 0.6$) (Fig. 6b, dashed line). Data: □, 300 ppm, 2 weeks; ■, 4 weeks; ●, 8 weeks; +, 16 weeks, Cronkite et al. [6] and ▾, 300 ppm, 16 weeks, Cronkite et al. [7].

reproduces the experimental data, indicating that saturation is likely to exist for benzene hematotoxicity.

Additional simulations (figures not shown) covering a dose-range from 1 to more than 4000 ppm generated model curves that fit the experimental data satisfactorily.

Recovery after long-term benzene intoxication

The model curve generated assuming no residual damage agrees well with the measurements of CFU-S in the case of a 300-ppm exposure for 2 and 4 weeks (Fig. 6a, solid line). However, extending the exposure to benzene for 8 and 16 weeks results in a considerable difference between the experimentally determined delayed recovery of CFU-S and the rapid recovery of the model stem cells (Fig. 6b, solid line). Therefore, modifications of the a_s:T_s ratio and p_s^{max} respectively were investigated for their effect on CFU-S recovery. It turned out that a considerable reduction of p_s^{max} only could explain the observed behaviour since it led to a satisfying reproduction of the data (Fig. 6b, dashed line).

Discussion

Two different theoretical methods, Time-Curve and Steady-State Analysis, were applied to analyze the response of murine hematopoiesis to in vivo benzene exposure of 100, 300 and 400 ppm.

Time-Curve Analysis, i.e., comparison of theoretical and experimental curves with respect to time course characteristics, revealed that erythropoietic cells were the most sensitive with respect to benzene intoxication and that the sensitivity of CFU-S ranged between that for erythro- and that for granulopoietic cells. For erythropoiesis, these findings confirm the conclusions already drawn from the experimental data [10,13,32], but this is less clear for granulopoietic cells and stem cells.

For example, Lee et al. [19], using the appearance of ^{59}Fe in circulating erythrocytes as an index of red cell development, concluded that benzene did not affect stem cells but selectively damaged pronormoblasts and normoblasts. However, our results strongly suggest that stem cells as well as erythropoietic progenitors are affected by benzene/-metabolites.

Considering only the experimental data for granulopoietic cells makes it difficult to decide whether or not they are target cells of benzene. In this case, model analysis is useful as it considers the interaction of the granulopoietic lineage with the other cell stages (erythropoietic and stem cells). A toxic effect on granulopoiesis is most probable from model considerations as the experimental data could be reproduced only by assuming a moderate damage to granulopoietic cells. Experimental data may not always reflect a clear reduction of cell numbers because of compensatory increases in cell production triggered by the benzene-induced loss of cells.

In a second step, experimental data were analyzed by Steady-State Analysis, which compares experimental steady-state cell numbers with model steady-state curves generated assuming various model toxicity patterns. In contrast to Time-Curve Analysis, this innovative method of data abstraction is useful in identifying the benzene-specific toxicity pattern independent of experimental dose and time course and requires only a minimum of readily obtainable information (e.g., steady-state

values for erythropoietic and granulopoietic precursors). Both analyses identified the same toxicity patterns and thus represent complementary tools for the examination of hematotoxic substances.

Cronkite et al. [7] reported large differences in the hematologic effects of inhalation of benzene at 316 ppm for 19 days vs. 3000 ppm for 2 days, although both regimes represented the same cumulative dose (concentration × time product). These results can be reconciled with the aid of the model dose-response curve. The curve asymptotically approaches the maximum loss coefficient in the range 300–600 ppm (Fig. 4). Therefore, the difference between the loss coefficients (k_S, k_E, k_G) for 316 vs. 3000 ppm is quite small and the tenfold increase in benzene dose results in only a 20% increase in cell loss per hour (316 ppm: $k_E = k_S = 1.37 \times 10^{-2}\ h^{-1}$, $k_G = 0.69 \times 10^{-2}\ h^{-1}$; 3000 ppm: $k_E = k_S = 1.6 \times 10^{-2}\ h^{-1}$, $k_G = 0.8 \times 10^{-2}\ h^{-1}$). Clearly the difference in exposure period in this experiment was more important than the difference in dose.

On the other hand, Green et al. [11,12] compared the effects of 103 ppm benzene delivered 6 h/day for 5 days vs. 9.6 ppm benzene delivered 6 h/day for 10 weeks (5 days/week). Whereas severe toxicity was observed following the 5-day, 103-ppm exposure, no toxicity in bone marrow and blood was observed following the 10-week (5 days/week) 9.6-ppm exposure. The difference among loss coefficients of these doses is large with the tenfold difference of the dose resulting in a considerable increase of loss coefficients (9.6 ppm: $k_E = k_S = 0.0164 \times 10^{-2}\ h^{-1}$, $k_G = 0.0082 \times 10^{-2}\ h^{-1}$; 103 ppm: $k_E = k_S = 0.486 \times 10^{-2}\ h^{-1}$, $k_G = 0.243 \times 10^{-2}\ h^{-1}$). In this case the more intense exposure over a shorter period was more effective than the less intense exposure over a longer period.

The mathematical equation representative of the dose-response relationship indicates saturation of benzene-induced hematotoxicity at doses in excess of 300 ppm. Since this most likely represents saturation of benzene metabolism and uptake [30], we conclude that the cumulative dose concept does apply for benzene intoxication with restriction. Depending on it's position with regard to saturation a greater dose × time product does not necessarily have a greater effect. Cumulative dose is not predictive of effect. Here, the model analysis enables one to make reasonable predictions.

The recovery of hematopoiesis after termination of chronic benzene intoxication (exposure period >8 weeks) is characterized by a substantial delay in CFU-S recovery. This has been demonstrated experimentally for doses of 300 and 400 ppm [6–8]. We were able to reproduce this recovery pattern assuming a severe reduction of the self-renewal probability of CFU-S. The result indicates that residual damage to the hemopoietic system manifests itself in an impaired self-renewal capacity of CFU-S. This model result agrees well with the results by Harigaya et al. [13] who demonstrated that benzene inhalation produced diminished stem cell replication. Since data for shorter exposure periods of 2 and 4 weeks at 300 ppm could be reproduced without assuming a compromised self-renewal capacity in the model, we conclude that the residual damage depends on the duration of the exposure period. Since no data exist on CFU-S recovery from smaller doses, the relationship between dose and residual injury remains open and a potential area for further investigation.

Considerable uncertainties exist concerning the behaviour of CFU-E to benzene

exposure. Baarson et al. [3] as well as Seidel et al. [32] showed that benzene exposures of 10, 300 or 900 ppm reduced CFU-E to significantly lower levels than BFU-E, but were reflected in only modest decreases in circulating red cells (see Fig. 5). Seidel et al. therefore concluded that CFU-E were the most sensitive of the erythropoietic cells [32] and that there was an enhanced cell flux through the CFU-E compartment [33], respectively. To test these assumptions, additional model calculations were performed assuming (i) a higher sensitivity of CFU-E and (ii) an increased cell flux of CFU-E (modeled by lowering CFU-E transit time). Both assumptions suitably explained the behaviour of CFU-E, but not that of the other erythropoietic cell stages (BFU-E, precursors and erythrocytes). Therefore, different explanations must be explored, e.g., interference of benzene metabolites with CFU-E receptors such that in vitro stimulating molecules are ineffective, as discussed by Cronkite et al. [7].

Finally, we would like to emphasize that considerable variations (beyond normal experimental variability) exist in the experimental data among different studies as well as among individual sets of data. Two reasons may possibly account for this phenomenon. First, it has been reported that a single genetic difference in the Ah-locus results in striking differences in benzo[*a*]pyrene-induced hematotoxicity [2,20,26]. This genetic difference also influences benzene metabolism and hematotoxicity [24,25,39,43]. With respect to the Ah-locus, we assigned the benzene inhalation studies to two distinct groups (use of 'responder' and 'non-responder' mice, respectively) and found that the variations of the experimental data within each of the two groups were reduced [31].

Second, model simulations which took exposure regimen into account (6 h/day, 5 days/week, mimicry of the work day), led to marked time-dependent oscillations (e.g., $\pm 20\%$) of different cell stages, that agreed with those fluctuations experimentally observed (Cronkite et al. [8]). Thus, the model analysis indicates that the exposure regimen might exert a considerable influence on the course of the experimental data.

In summary, the mathematical methods reported herein provide efficient tools for the analysis of benzene-induced hematotoxicity. They enable the identification and quantification of toxic effects as well as comparison of different toxicity concepts. Moreover, they are suitable for the analysis of any hematotoxic agent in general. The use of this approach permits the testing of alternate hypotheses with exclusion of unsuitable ones and optimization of experiments, which potentially may avoid unnecessary animal experimentation.

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References

1 P.L. Altmann and D.D. Katz, Inbred and Genetically Defined Strains of Laboratory Animals, Part 1, Fed. Am. Soc. Exp. Biol., 1979.

2 V. Anselstetter and H. Heimpel, Acute hematotoxicity of oral benzo[*a*]pyrene: The role of the Ah-locus. Acta Haematol., 76 (1986) 217.

3 K.A. Baarson, C.A. Snyder and R.E. Albert, Repeated exposure of C57BL mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. Toxicol. Lett., 20 (1984) 337.

4 K.A. Baarson, C.A. Snyder, J.D. Green, A. Sellakumar, B.D. Goldstein and R.E. Albert, The hematotoxic effects of inhaled benzene on peripheral blood, bone marrow and spleen cells are increased by ingested ethanol. Toxicol. Appl. Pharmacol., 64 (1982) 393.

5 H.S. Cohen, M.L. Freedman and B.D. Goldstein, The problem of benzene in our environment: Clinical and molecular considerations. Am. J. Med. Sci., 275 (1978) 124.

6 E.P. Cronkite, R.T. Drew, T. Inoue and J.E. Bullis, Benzene hematotoxicity and leukemogenesis. Am. J. Ind. Med., 7 (1985) 447.

7 E.P. Cronkite, R.T. Drew, T. Inoue, Y. Hirayabashi and J.E. Bullis, Hematotoxicity and carcinogenicity of inhaled benzene. Environ. Health Perspect., 82 (1988) 97.

8 E.P. Cronkite, T. Inoue, A.L. Carsten, M.E. Miller, J.E. Bullis and R.T. Drew, Effects of benzene inhalation on murine pluripotent stem cells. J. Toxicol. Environ. Health., 9 (1982) 411.

9 D.P. Gill and A.E. Ahmed, Covalent binding of (^{14}C) benzene to cellular organelles and bone marrow nucleic acids. Biochem. Pharmacol., 30 (1981) 1127.

10 D.P. Gill, V.K. Jenkins, R.R. Kempen and S. Ellis, The importance of pluripotential stem cells in benzene toxicity. Toxicology, 16 (1980) 163.

11 J.D. Green, C.A. Snyder, J. Lobue, B.D. Goldstein and R.E. Albert, Acute and chronic dose/response effect of benzene inhalation on the peripheral blood, bone marrow and spleen cells of CD-1 male mice. Toxicol. Appl. Pharmacol., 59 (1981) 204.

12 J.D. Green, C.A. Snyder, J. Lobue, B.D. Goldstein and R.E. Albert, Acute and chronic dose/response effect of benzene inhalation on multipotential hematopoietic stem (CFU-S) and granulocyte/macrophage progenitor (GM-CFU-C) cells in CD-1 male mice. Toxicol. Appl. Pharmacol., 58 (1981) 492.

13 K. Harigaya, M.E. Miller, E.P. Cronkite and R.T. Drew, The detection of in vivo hematotoxicity of benzene by in vitro liquid bone marrow culture. Toxicol. Appl. Pharmacol., 60 (1981) 346.

14 H. Herrmann and S. Dobbertin, Wirkungen auf das hämatopoetische System, in Umweltbundesamt, luftqualitätskriterien für Benzol (Berichte 6/82), Erich Schmidt Verlag, Berlin, 1982, p. 26.

15 R.L. Hilderbrand and M.G. Murphy, The effects of benzene inhalation on murine hematopoietic precursor cells (CFU-E, BFU-E and CFU-GM). Int. J. Cell Cloning, 1 (1983) 240.

16 G.F. Kalf, Recent advances in the metabolism and toxicity of benzene. CRC, Crit. Rev. Toxicol., 18 (1987) 141.

17 G.F. Kalf, T.H. Rurhmore and R. Snyder, Benzene inhibits RNA synthesis in mitochondria from liver and bone marrow. Chem.-Biol. Interact., 42 (1982) 353.

18 G.F. Kalf, R. Snyder and T.H. Rushmore, Inhibition of RNA synthesis by benzene metabolites and their covalent binding to DNA in rabbit bone marrow mitochondria in vitro. Am. J. Ind. Med., 7 (1985) 485.

19 E.W. Lee, J.J. Kocsis and R. Snyder, Acute effect of benzene on ^{59}Fe-incorporation into circulating erythrocytes. Toxicol. Appl. Pharmacol., 27 (1974) 431.

20 C. Legraverend, D.E. Harrison, F.W. Ruscetti and D.W. Nebert, Bone marrow toxicity induced by oral benzo(*a*)pyrene: Protection resides at the level of the intestine and the liver. Toxicol. Appl. Pharmacol., 70 (1983) 390.

21 B.K. Leong, Experimental benzene intoxication. J. Toxicol. Environ. Health, Suppl. 2 (1977) 45.

22 M. Loeffler, K. Pantel, H. Wulff and H.E. Wichmann, A mathematical model of erythropoiesis in mice and rats. Part 1: Structure of the model. Cell Tissue Kinet., 22 (1989) 13.

23 S.L. Longacre, J.J. Kocsis, C.M. Witmer, E.W. Lee, D. Sammett and R. Snyder, Toxicological and biochemical effects of repeated administration of benzene in mice. J. Toxicol. Environ. Health, 7 (1981) 223.

24 S.L. Longacre, J.J. Kocsis and R. Snyder, Influence of strain differences in mice on the metabolism and toxicity of benzene. Toxicol. Appl. Pharmacol., 60 (1981) 398.

25 C.A. Luke, R.R. Tice and R.T. Drew, The effect of expusure regimen and duration on benzene-induced bone marrow damage in mice II. Strain comparisons involving B6C3F1 C57BL/6 and DBA/2 male mice. Mutat. Res., 203 (1988) 273.

26 D.W. Nebert, N.M. Jensen, R.C. Levitt and J.S. Felton, Toxic chemical depression of the bone marrow and possible aplastic anemia explainable on a genetic basis. Clin. Toxicol., 16 (1980) 99.

27 D.W. Nebert, R.C. Levitt, N.M. Jensen, G.H. Hambert and J.S. Felton, Birth defects and aplastic anemia: Differences in polycyclic hydrocarbon toxicity associated with the Ah-locus. Arch. Toxicol., 39 (1977) 109.

28 G.J. Rosenthal and C.A. Snyder, The effects of ethanol and the role of the spleen during benzene-induced hematotoxicity. Toxicology, 30 (1984) 283.

29 T. Rushmore, R. Snyder and G. Kalf, Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria in vitro. Chem.-Biol. Interact., 49 (1984) 133.

30 P.J. Sabourin, B.T. Chen, G. Lucier, L.S. Birnbaum, E. Fisher and R.F. Henderson, Effect of dose on the absorption and excretion of (^{14}C)-benzene administered orally or by inhalation in rats and mice. Toxicol. Appl. Pharmacol., 87 (1987) 325.

31 S. Scheding, Benzene, Benzo[*a*]pyrene, Lead: Model analysis of the hematotoxic effects of three important pollutants, Thesis, University of Cologne, 1990, p. 1.

32 H.J. Seidel, E. Barthel and D. Zinser, The hemopoietic stem cell compartments in mice during and after long-term inhalation of three doses of benzene. Exp. Hematol., 17 (1989) 300.

33 H.J. Seidel, G. Beyvers, M. Pape and E. Barthel, The influence of benzene on the erythroid cell system in mice. Exp. Hematol., 17 (1989) 760.

34 H.J. Seidel, D. Zinser, M. Pforr and G. Beyvers, Benzene inhalation and hemopoietic stem cells in mice. Exp. Hematol. 15 (1987) 518.

35 S. Schmitz, M. Loeffler, J.B. Jones, R.D. Lange and H.E. Wichmann, Synchrony of bone marrow proliferation and maturation as the origin of cyclic haemopoiesis. Cell Tissue Kinet., 23 (1990) 425.

36 C.A. Snyder, K.A. Baarson, B.D. Goldstein and R.E. Albert, Ingestion of ethanol increases the hematotoxicity of inhaled benzene in C57BL mice. Bull. Environ. Contam. Toxicol., 27 (1981) 175.

37 C.A. Snyder, B.D. Goldstein and A. Sellakumar, Hematotoxicity of inhaled benzene to Sprague–Dawley rats and AKR mice at 300 ppm. J. Toxicol. Environ. Health, 4 (1978) 605.

38 C.A. Snyder, B.D. Goldstein, A. Sellakumar, I. Bromberg, S. Laskin and R.E. Albert, Toxicity of chronic benzene inhalation in CD-1 mice exposed to 300 ppm. Bull. Environ. Contam. Toxicol., 29 (1982) 385.

39 C.A. Snyder, B.D. Goldstein, A.R. Sellakumar, I. Bromberg, G. Laskin and R.E. Albert, The inhalation toxicity of benzene: incidence of hematopoietic neoplasmas and hematotoxicity in AKR/J and C57BL/6J mice. Toxicol. Appl. Pharmacol., 54 (1980) 323.

40 C.A. Snyder, J.D. Green, J. LoBue, B.D. Goldstein and R.E. Albert, Protracted benzene exposure causes a proliferation of myeloblasts and/or promyelocytes in CD-1 mice. Bull. Environ. Contam. Toxicol., 27 (1981) 17.

41 R. Snyder and J. J Kocsis, Current concepts of chronic benzene toxicity. CRC, Crit. Rev. Toxicol., (1975) 256.

42 R. Snyder, E.W. Lee and J.J. Kocsis, Binding of labeled benzene metabolites to mouse liver and bone marrow. Res. Commun. Chem. Pathol. Pharmacol., 20 (1978) 191.

43 R. Snyder, S.L. Longacre, C.M. Witmer and J.J. Kocsis, Metabolic correlates of benzene toxicity, in R. Snyder, D.V. Parke, J.J. Kocsis, D.J. Jollow, C. Gordon Gibson and C.M. Witmer (Eds.), Biological Reactive Intermediates II. Chemical Mechanisms and Biological Effects, Part A, Plenum Press, New York/London, 1982, p. 245.

44 E.M. Uyeki, A. Ashkar, T. Shoeman and U. Bisel, Acute toxicity of benzene inhalation to hemopoietic precursor cells. Toxicol. Appl. Pharmacol., 40 (1977) 49.

45 H.E. Wichmann and M. Loeffler, Mathematical modeling of cell proliferation. Stem cell regulation in hemopoiesis, Vols. I and II, CRC Press, Boca Raton, 1985.

46 H.E. Wichmann, M. Loeffler and S. Schmitz, A concept of hemopoietic regulation and its biomathematical realization. Blood Cells, 14 (1988) 411.