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Assessment of Myelotoxicity Caused by Environmental Chemicals

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Potential antineoplastic agents must be screened for the delayed toxicity that occurs in many cases of drug-induced bone marrow aplasia. *In vitro* clonal assays for hematopoietic progenitor cells have been developed to assess the degree of myelotoxicity. This adverse side effect is often the limiting factor in the development of new cancer chemotherapeutics. In addition, many environmental chemicals are cytotoxic to rapidly proliferating cells, but a systematic assessment of their myelotoxicity has not been performed. We have used clonal marrow assays to investigate a panel of chemicals including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, polybrominated biphenyls, diethylstilbestrol, benzo(a)pyrene and indomethacin. All were immunotoxic, some to pleuripotent hemopoetic stem cells and others to granulocyte-macrophage progenitors, and at concentrations below those causing other toxic manifestations. This shows that these bone marrow clonal assays, and hopefully future ones for erythroid, B- and T-lymphocytes, and megakaryocytes, will provide the specificity and sensitivity necessary to delineate the myelotoxicity of a broad spectrum of environmental chemicals.

Introduction

As one of the most rapidly renewing cell populations, hemopoietic cells have proven very sensitive to cytotoxic agents (*1-5*). Bone marrow failure is a significant complication of cancer chemotherapy and has also been implicated as a result of exposure to numerous drugs (*6-9*) and environmental agents (*10-12*). During the past decade, a variety of semisolid *in vitro* culture techniques have been developed for most of the hemopoietic cell lines. Examination of colony formation of the hemopoietic cells following exposure to various agents has proven to be a sensitive indicator of toxicity as well as a means for mechanistic study of the toxicity of various drugs (*9*).

Murine pleuripotent hemopoietic stem cells can be detected by injecting bone marrow cells into irradiated recipients and counting the number of colonies that form in 8 days (*13*). More differentiated committed progenitor cells can be induced to form colonies in semisolid media by adding appropriate stimulating factors. Currently, clonal assay procedures exist (Fig. 1) for B- and T-lymphocytes, macrophage-granulocyte progenitors, megakaryocytes, eosinophils and erythroid precursors (*14,15*). Colonies arise from proliferation of individual cells and the number of colony forming cells are altered under abnormal physiological conditions following treatment with certain drugs, and in certain disease states such as chronic myelogenous leukemia and polycythemia rubra vera (*15*).

Hemopoietic stem cells appear to have a limited capacity for division. This can be demonstrated by serial passage of bone marrow cells into irradiated recipients (*16*). Serial transfer of the donor cells will permit the marrow tissue to survive beyond the normal life expectancy and shows that bone mar-

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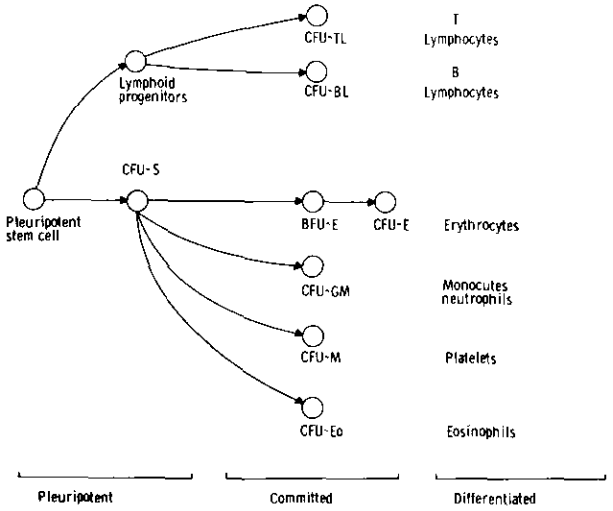

FIGURE 1. Model of hematopoiesis as determined by *in vitro* and *in vivo* clonal assays. CFU-S, colony-forming unit-spleen; CFU-TL and CFU-BL, colony-forming unit-T and B-lymphocyte, respectively; BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid; CFU-GM, colony-forming unit-granulocyte-macrophage; CFU-M, colony-forming unit-megakaryocyte; CFU-Eo, colony-forming unit-eosinophil.

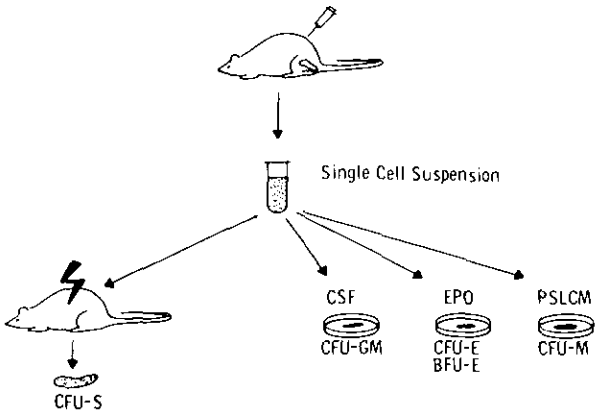

FIGURE 2. Scheme of testing for myelotoxicity. Following exposure to a chemical a single cell suspension is made of femoral marrow. Following enumeration of nucleated marrow cells they are injected into irradiated recipients to measure colony-forming units-spleen (CFU-S) and plated in *in vitro* cultures to which has been added colony-stimulating factor (CSF), erythropoietin (EPO) or pokeweed stimulated lymphocyte culture medium (PSLCM) which will allow the proliferation of granulocyte-macrophage progenitors (CFU-GM), erythroid progenitors (BFU-E and CFU-E) and megakaryocytes (CFU-M), respectively.

row would not be a limiting factor in aging of the mouse. Fetal or embryonic hemopoietic tissue has a greater capacity for serial transfer than from adult mice. However, serial transfer capacity changes very little with aging, suggesting the stem cell compartment is minimally used during adult life (*18*). Several studies have shown that exposure to a toxic agent such as busulfan can induce a permanent stem cell defect that persists throughout the life of the animal without any evidence of recovery (*19,20*). Animals with permanent stem cell defect may have normal bone marrow cellularity and peripheral blood counts, but the residual injury can be demonstrated by increased sensitivity to irradiation (*19*), decreased erythropoietic response following anemia stress (*21*), decreased proliferative capacity of bone marrow or after serial transplantation (*22*) and decreased antibody response (*20*). These animals will eventually die of chronic aplastic marrow failure (*23*).

The concept of residual marrow injury may prove to be very important in toxicology testing. Currently toxicological assessment devotes little effort to understanding myelotoxicity and the usual screening programs would not detect residual stem cell injury since these animals may appear clinically normal. Chronic aplastic marrow failure, frequently a fatal syndrome, has a history of drug or chemical exposure in approximately 50% of the cases (*23*). Although marrow failure is rare, the studies cited above suggest a much larger population may exist with undetected residual stem cell injury following chemical exposure. This population would show decreased ability to respond to situations requiring increased hemopoietic cell production and may be more susceptible to other cytotoxic drugs. While the model of induced stem cell defect and marrow failure was developed in mice, it would appear relevant to man, since it has been shown that patients exposed to chemotherapy show a prolonged decrease in bone marrow colony-forming cells in spite of normal peripheral blood counts, and it has been suggested that the marrow is more fragile in these patients (*24*). Based on the mouse model, these patients would be expected to have less bone marrow reserve when extra hematopoietic cells are needed (Fig. 2).

Assessment of Bone Marrow Capacity by Use of Clonal Assays

Pleuripotent Stem Cells (CFU-S)

Till and McCulloch (*13*) showed that when mouse marrow cells were injected into heavily irradiated

recipients, discrete nodules of hemopoietic cells would form in the spleen of the recipients in 8 or 9 days. The nodules, called spleen colonies, contained pure or mixed populations of various hemopoietic cell lines (*15*) and were shown by chromosomal markers (*25*) to arise from individual cells which have been called colony-forming unit-spleen (CFU-S), or pleuripotent stem cells.

When isolated, made into a suspension and reinjected into an irradiated recipient, a stem cell colony containing cells of one hemopoietic cell line would give rise to new colonies of all cell lines, demonstrating both the self-renewal capacity and pleuripotentiality of the CFU-S. The relationship of the CFU-S to lymphocytes, an area of controversy, now appears to be resolved with the CFU-S and lymphoid progenitors being derived from a common, more primitive, stem cell (*15,26*). Most CFU-S have a long generation time or remain longer in the G_0 cell cycle phase. When marrow depletion occurs, CFU-S respond by more rapid proliferation and differentiation. As mentioned earlier, CFU-S have a finite self-replication capacity which under normal circumstances exceeds the life span of the mouse. Chemotherapy or irradiation can damage the replicative capacity of CFU-S with bone marrow failure as a late sequela.

Granulocyte-Macrophage Progenitors (CFU-GM)

Shortly after the development of the CFU-S assay, Bradley and Metcalf (*27*) as well as Pluznik and Sachs (*28*) demonstrated that bone marrow cells would, with appropriate stimuli, form discrete colonies in semisolid media *in vitro*. These colonies arose from individual cells, and the cells giving rise to these colonies were designated colony-forming units in culture or CFU-C. The proliferation of these cells requires the presence of a colony-stimulating activity (CSF), and in 7 days colonies containing both granulocytes and monocyte-macrophages are formed. A single cell can give rise to both granulocytes and macrophages (*29*), and this cell has subsequently been renamed CFU-GM for colony-forming unit, granulocyte-macrophage. The CFU-GM is a separate cell population from the CFU-S, has a relatively high proliferation rate and little capacity for self-renewal (*15*). Conditions that demand granulopoiesis result in increased serum and tissue CSF levels as well as increased marrow CFU-GM numbers while cytotoxic agents cause decreased CFU-GM numbers (*30*). The CFU-GM assay is available for most mammalian species including man and has led to the identification of factors having both positive and negative effects on granulopoiesis.

Erythroid Progenitors (CFU-E, BFU-E)

More recently, it has been reported that the addition of erythropoietin to bone marrow cultures will stimulate the growth of colonies that contain hemoglobulin (*31*). Further study has shown that these erythroid colonies contain at least two classes of stem cells, a more primitive erythroid burst-forming unit (BFU-E) requiring long culture periods (10-14 days) with high erythropoietin levels and a more differentiated colony-forming unit-erythroid (CFU-E) requiring lower erythropoietin level and only 2 days of culture (*23,33*). Marrow CFU-E numbers follow a predictive pattern, increased following bleeding (*34*) and decreased during plethora induced by red cell transfusions (*35*). Erythroid progenitors as measured by clonal assays are sensitive to cytostatic agents (*30,36,37*) and known marrow toxicicants such as benzene (*38,39*). Use of these assays has revealed that some patients with aplastic anemia possess a population of lymphocytes capable of suppressing *in vitro* erythroid-colony formation by normal human bone marrow (*40*), whereas in congenital hypoplastic (Diamond-Blackfan) anemia, lymphocytes are normal, and the defect appears to reside in the erythroid progenitors (*41*). Thus these assays allow not only study of erythroid progenitor cell numbers but can also contribute to our understanding of hormonal factors and cell to cell interaction as may occur in disease states. Another technique used to study erythropoiesis is ^{59}Fe uptake in spleen and marrow which provides a specific marker for hemoglobin synthesis (*15,36*). In this assay, mice are killed 18 hr after injection of the isotope and tissues collected for counting. Further studies are needed, however, to determine the correlation between ^{59}Fe uptake and the clonal assays for erythroid precursors.

Other Clonal Bone Marrow Assays (CFU-BL, CFU-TL, CFU-M)

Clonal marrow assays have been developed for B-lymphocytes (CFU-BL), T-lymphocytes (CFU-TL), and megakaryocytes (CFU-M) by adding appropriate stimuli to semi-solid marrow cultures (*15,38-40*). These new model systems should allow further understanding of granulopoiesis and may represent sensitive indicators of toxicity but need to be further examined.

Effects of Environmental Agents on Myelotoxicity

Benzene is one of the more widely known myelotoxic agents where short term exposure will induce profound pancytopenia and anemia (*41-43*). Following benzene exposure, there is decreased ^{59}Fe uptake by hematopoietic organs (*41*), but whether this compound acts at the more primitive BFU-E or the more mature CFU-E has not been defined.

Mercury has been reported to cause pancytopenia in man. *In vitro* studies have shown that micromolar concentrations of both inorganic and organic mercury inhibited colony formation in primary culture of mouse bone marrow (*46*). Addition of a toxic chemical to marrow cultures may suggest myelotoxicity but further confirmation by *in vivo* studies are necessary.

We have used a mouse model to investigate immunotoxicity. Our general experimental design employs the female B6C3F1 hybrid mouse exposed to chemicals at sublethal concentrations and fractions thereof. A series of cell and humoral mediated immunoassays, in addition to clonal marrow assays are performed. This review deals principally with the latter by describing the effects of a variety of environmental pollutants on the pleuripotent hemopoietic stem cell (CFU-S) and the granulocyte-macrophage progenitor (CFU-GM).

Tetrachlorodibenzo-*p*-dioxin (TCDD) is an environmental pollutant that is ubiquitous, persists in the environment, and causes varying degrees of marrow hypocellularity in all species of animals studied to date (*47*). Mice were exposed to 0, 1.0, 5.0 or 15 μg/kg body weight of TCDD pre- and postnatally by maternal dosing (*48*). As shown in Table 1, both 5 and 15 μg/kg dosage groups showed a significant reduction in marrow cellularity, CFU-S and CFU-GM. Hematology profiles and blood smears revealed a normocytic anemia in these mice (*48*); bone marrow toxicity was correlated with depressed immunologic and host resistance responses.

Polybrominated biphenyls (PBB) were accidently introduced in cattle and chicken feed in Michigan and resulted in widespread human exposure (*49*). Mice were exposed pre/postnatally by maternal dosing at 0, 1, 3 and 10 mg/kg maternal body weight (*49*). As shown in Table 1, there was no significant decrease in marrow cellularity or CFU-GM. The CFU-S in male mice at the highest dose was 80% of controls but the difference was not significant (*50*). At the 1 mg/kg level, the CFU-GM numbers were significantly enhanced. In general the study showed little or no marrow alterations and also only minimal alterations in immunological function tests.

Benzo(a)pyrene, a constituent of petroleum compounds and derivatives, has long been recognized as a potent mutagen and carcinogen. Mice were exposed to 50, 200 or 400 mg/kg benzo(a)pyrene by subcutaneous injection for 10 doses over a 14-day period. Bone marrow cellularity showed a significant increase but there was a significant and dose responsive reduction in both marrow CFU-S and CFU-GM (Table 1). These mice also showed a vari-

Table 1. The effect of environmental chemicals on bone marrow.

Chemical	Dose, mg/kg	Cell or colony numbers, % of controls		
		Marrow cellularity	Pleuripotent stem cells (CFU-S)	Granulocyte-macrophage progenitors (CFU-GM)
TCDD	0.001	100[a]	ND	121
	0.005	83[b]	68[b]	90
	0.015	61[c]	37[c]	21[c]
PBB	1	91	ND	125[b]
	3	95	ND	111
	10	86	ND	108
B(a)P	50	115	80[c]	85
	200	105	80[c]	82[b]
	400	120[b]	51[c]	59[c]
DES	0.2	84	97	16[b]
	2	74	88	65[c]
	8	68	59[c]	63[c]
IND	1	112	108	118
	2	88	ND	122
	4	96	81	120

[a]Percent of controls, 7 mice/group.
[b]$P < 0.05$ vs. controls.
[c]$P < 0.01$ vs. controls.

ety of immunological defects, primarily of humoral immunity (*51*).

Diethylstilbestrol (DES) was formerly prescribed as a synthetic estrogen and also was used to fatten cattle. Human residues accumulated from ingestion of dairy and meat products have been found. Mice were exposed by subcutaneous injection to 0.2, 2 and 8 mg/kg body weight for 5 consecutive days (*52*). There was a dramatic decrease in marrow cellularity, CFU-S and CFU-GM (Table 1). These animals also exhibited highly enhanced sensitivity to *Listeria monocytogenes* and other generalized immunotoxic responses (*53*).

Indomethacin (IND) is a potent anti-inflammatory agent that appears to act by inhibiting prostaglandin synthetase. Mice were exposed to 0, 1, 2 and 4 mg/kg by subcutaneous injection for six consecutive days. In general there tended to be some enhancement of CFU-GM with no significant alterations in marrow cellularity or CFU-S (Table 1). These animals showed enhanced resistance to *Listeria monocytogenes* (*54*).

Mice exposed to orthophenylphenol or tris(2,3-dichloropropyl) phosphate exhibited no alteration in bone marrow cellularity or CFU-GM numbers (*55*).

Conclusions

Clonal marrow assays have greatly aided our understanding of hematopoiesis and factors controlling the production of blood cells. These assays are currently finding widespread use in cancer chemotherapy centers as tools to understand and to monitor myelotoxicity following antineoplastic therapy. We have shown that some environmental pollutants likewise have effects on marrow cellularity and progenitor cells and that these effects are readily detected by clonal marrow assays. It would appear that wider application of these techniques can help define myelotoxicity resulting from chemical exposure.

Anemia is commonly reported in long term chronic toxicity tests. This would suggest that myelotoxicity assessment panels should also include assays for erythroid progenitors. Erythropoiesis can also be assessed by *in vitro* clonal assays or by ^{59}Fe uptake *in vivo*.

Recently it has been shown that hemopoietic stem cells have a limited renewal capacity and that damage to these cells can induce a permanent decrease in their proliferative capacity (*20*). This residual marrow damage would not be predicted by parameters such as bone marrow cellularity, peripheral blood counts or even in some cases by CFU-S numbers (*20,36*). However "stressors" such as serial transfer of marrow (*20*), anemia (*21*) or irradiation (*19*) have revealed that these mice have a more limited hemopoietic response than controls. With the plethora of environmental chemicals it seems possible that exposure to one chemical may induce residual marrow damage that would make an individual more susceptible to a second chemical insult, although this hypothesis remains to be tested.

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REFERENCES

1. Delmonte, L. Effect of myleran on murine hemopoiesis. I. Granulocytic cell lines specificity of action on progenitor cells. Cell Tissue Kinet. 11: 347-358 (1978).
2. Jenkins, V. K., Costanzi, J. J., and Ellis, H. N. Effects of single and combined chemotherapeutic agents on hemopoietic stem cells in mice. Am. J. Hematol. 1: 79-88 (1976).
3. Brown, C. H., and Carbone, P. P. Effect of chemotherapeutic agents on normal mouse bone marrow grown *in vitro*. Cancer Res. 31: 185-190 (1971).
4. Haas, R. J., Rohruber, W., Netzel, B., and Lau, E. Effect of CCNU on hematopoiesis in rats. Cancer Treatment Repts. 63: 377-383 (1979).
5. Dumenil, D., Sainteny, F., and Frindel, E. Some effects of chemotherapeutic drugs on bone marrow stem cells. Cancer Chemother. Pharmacol. 2: 197-207 (1979).
6. McVie, J. G. Drug induced thrombocytopenia. In: Blood Disorders Due to Drugs and Other Agents, R. H. Girdwood, Ed., Excerpta Medica, Amsterdam, 1973.
7. Druart, F., Frocrain, C., Metois, P., Martin, J., and Matuchansky, C. Association of cimetidine and bone marrow suppression in man. Am. J. Dig. Dis. 24: 730-731 (1979).
8. Miller, A. M., Gross, M. A., and Yunis, A. Heterogeneity of human colony-forming cells (CFUs) with respect to their sensitivity to chloranphenicol. Exptl. Hemat. 8: 236-242 (1980).
9. Maurer, H. R., and Henry, R. Drug evaluation on haemopoietic cells *in vitro*. I. A micro agar colony assay with semi-automatic optical monitoring. Drug Res. 28: 601-605 (1978).
10. Strom, S., Johnson, R. L., and Uyeki, E. M. Mercury toxicity to hemopoietic and tumor colony-forming cells and its reversal by selenium *in vitro*. Toxicol. Appl. Pharmacol. 49: 431-436 (1979).
11. Irons, R. D., Heck, H. d'A., Moore, B. J., and Muirhead, K. A. Effects of short-term benzene administration on bone marrow cell cycle kinetics in the rat. Toxicol. Appl. Pharmacol. 51: 399-409 (1979).
12. Snyder, C. A., Goldstein, B. D., Sellakumar, A. R., Bromberg, I., Laskin, S., and Albert, R. E. The inhalation toxicology of benzene: Incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. Toxicol. Appl. Pharmacol. 54: 323-331 (1980).
13. Till, J. E., and McCulloch, E. A. A direct measurement on the radiation sensitivity of normal mouse bone marrow cells. Radiation Res. 14: 213-222 (1961).

14. Cline, M. J., and Golde, D. W. Controlling the production of blood cells. Blood 53: 157-165 (1979).
15. Quesenberry, P., and Levitt, L. Hematopoietic stem cells. New Engl. J. Med. 301: 755-760, 819-823, 868-872 (1979).
16. Metcalf, D., and Moore, M. A. S. Haemopoietic cells. In: Frontiers of Biology, A. Nueberger and E. L. Tatum, Eds., North Holland, Amsterdam, 1977.
17. Vos, O., and Dolmans, M. J. A. S. Self-renewal of colony forming units (CFU) in serial bone marrow transplantation experiments. Cell Tissue Kinet. 5: 371-385 (1972).
18. Botnick, L. E., Hannon, E. C., and Hellman, S. Nature of the hemopoietic stem cell compartment and its proliferative potential. Blood Cells 5: 195-210 (1979).
19. Fried, W., and Barone, J. Residual marrow damage following therapy with cyclophosphamide. Exptl. Hemat. 8: 610-614 (1980).
20. Botnick, L. E., Hannon, E. C., and Hellman, S. Multisystem stem cell failure after apparent recovery from alkylating agents. Cancer Res. 38: 1942-1947 (1978).
21. Braunschweiger, P. G., Schenken, L. L., and Schiffer, L. M. Adriamycin-induced delayed erythropoietic injury expressed following anemia stress. Cancer Res. 40: 2257-2262 (1980).
22. Botnick, L. E., Hannon, E. C., and Hellman, S. Limited proliferation of stem cells surviving alkylating agents. Nature 262: 68 (1976).
23. Morley, A., and Blake, J. An animal model of chronic aplastic marrow failure. I. Late marrow failure after Busulfan. Blood 44: 49-56 (1979).
24. Hartmann, O., Parmentier, C., Lemerle, J., Gout, M., and Schweisguth, G. Sequential study of the bone marrow granulocytic progenitor cells (CFC) in children treated by chemotherapy for non-Hodgkin malignant lymphomas. Nouv. Rev. Franc. Hematol. 21: 239-250 (1979).
25. Becker, A. J., McCulloch, E. A., and Till, J. E. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 197: 452-455 (1963).
26. Abramson, S., Miller, R. G., and Phillips, R. A. The identification in adult bone marrow of pluripotent and restricted stem cells of the myeloid and lymphoid systems. J. Exptl. Med. 145: 1567-1579 (1977).
27. Bradley, T. R., and Metcalf, D. The growth of mouse bone marrow cells *in vitro*. Austral. J. Exptl. Biol. Med. Sci. 44: 287-300 (1966).
28. Plunznik, D. H., and Sachs, L. The cloning of normal "mast" cells in tissue culture. J. Cell. Physiol. 66: 319-324 (1965).
29. Moore, M. A. S., Williams, N., and Metcalf, D. Purification and characterization of the *in vitro* colony forming cells in monkey hemopoietic tissue. J. Cell. Physiol. 79: 283-292 (1972).
30. Hodgson, G. S., Bradley, T. R., Martin, R. F., Sumner, M., and Fry, P. Recovery of proliferating haemopoietic progenitor cells after killing by hydroxyurea. Cell Tissue Kinet. 8: 51-60 (1975).
31. McLeod, D. L., Shreeve, M. M., and Alexrad, A. A. Improved plasma culture system for production of erythrocytic colonies in vitro: quantitative assay method for CFU-E. Blood 44: 517-534 (1974).
32. Gregory, C. J. Erythropoietin sensitivity as a differentiation marker in the hemopoietic system: studies of three erythropoietic colony responses in culture. J. Cell. Physiol. 89: 289-301 (1976).
33. Hara, H., and Ogawa, M. Erythropoietic precursors in mice under erythropoietic stimulation and suppression. Exptl. Hematol. 5: 141-148 (1977).
34. Pannacciulli, I. M., Massa, A., Saviane, A. G., Bianchi, G., BogLiolo, G. V., and Ghio, R. Effect of bleeding on *in vivo* and *in vitro* colony-forming hemopoietic cells. Acta Haemat. 58: 27-33 (1977).
35. Spivak, J. L., and Graber, S. E. Erythropoietin and the regulation of erythropoiesis. Johns Hopkins Med. J. 146: 311-320 (1980).
36. Boggs, D. R., Boggs, S. S., Chervenick, P. A., and Patrene, K. D. Murine recovery from Busulfan-induced hematopoietic toxicity as assessed by three assays for colony-forming cells. Am. J. Hemat. 8: 43-54 (1980).
37. Udupa, K. B., and Reissmann, K. R. Cell kinetics of erythroid colony-forming cells (CFU-E) studied by hydroxyurea injections and sedimentation velocity profile. Exptl. Hemat. 6: 398-404 (1978).
38. Levin, J. L., Levin, F. C., and Metcalf, D. The effects of acute thrombocytopenia on megakaryocyte-CFC and granulocyte-macrophage-CFC in mice: studies of bone marrow and spleen. Blood 56: 274-283 (1980).
39. Claësson, M. H., Fiad, H.-D., and Opitz, H. G. B and T lymphocyte colony formation in agar: 2-mercaptoethanol-activated albumin can substitute for 2-mercaptoethanol. Cell. Immunol. 46: 398-404 (1979).
40. Metcalf, D., Nossal, G. J. V., Warner, N. L., Miller, J. F. A. P., Mandel, T. E., Layton, J. E., and Gutman, G. A. Growth of B-lymphocyte colonies *in vitro*. J. Exptl. Med. 142: 1534-1549 (1975).
41. Leong, B. K. J. Experimental benzene intoxication. J. Toxicol. Environ. Health Suppl. 1: 45-62 (1976).
42. Synder, C. A., Goldstein, B. D., Sellakumar, A., Wolman, S. R., Bromberg, I., Erlichman, M. N., and Laskin, S. Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J. Toxicol. Environ. Health 4: 605-618 (1978).
43. Johnston, R. V., Pinkerton, M. N., Mensik, D. C., Swaim, L. D., Linscombe, V. A., Benge, M. C., Barna-Lloyd, G., and Kilian, D. J. Hematologic and mylogenous effects of inhaled benzene in the pig and the rat. J. Toxicol. Environ. Health. 6: 1025-1035 (1979).
44. Hoffman, R., Zanjani, E. D., Lutton, J. D., Zalusky, R., and Wasserman, L. R. Suppression of erythroid-colony formation by lymphocytes from patients with aplastic anemia. New Engl. J. Med. 296: 10-13 (1977).
45. Nathan, D. G., Hillman, D. G., Chess, L., Alter, B. P., Clarke, B. J., Breard, J., and Housman, D. E. Normal erythropoietic helper T cells in congenital hypoplastic (Diamond-Blackfan) anemia. New Engl. J. Med. 298: 1049-1051 (1978).
46. Strom, S., Johnson, R. L., and Uyeki, E. M. Mercury toxicity to hemopoietic and tumor colony-forming cells and its reversal by selenium *in vitro*. Toxicol. Appl. Pharmacol. 49: 431-436 (1979).
47. McConnell, E. E., and Moore, J. A. Toxicopathology characteristics of the halogenated aromatics. Ann. N.Y. Acad. Sci. 77: 138-150 (1979).
48. Luster, M. I., Boorman, G. A., Dean, J. H., Harris, M. W., and Luebke, R. W. Examination of bone marrow, immunologic parameters and host susceptibility following pre- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TDCC). Int. J. Immunopharmac. 2: 301-310 (1980).
49. Humphrey, H. E. B., Haynes, N. S., and Budd, M. L. PBB analysis in dairy farmers and residents of the State of Michigan. In: Trace Substances in Environmental Health, Vol. IX, D. D. Hemphill, Ed., University of Missouri, Columbia, Mo., 1980.
50. Luster, M. I., Boorman, G. A., Harris, M. W., and Moore, J. A. Laboratory studies on polybrominated biphenyl-induced immune alterations following low-level chronic or pre/postnatal exposure. Int. J. Immunopharmac. 2: 69-80 (1980).
51. Dean, J. H., Luster, M. I., and Boorman, G. A. Effect of

benzo(a)pyrene on immunologic, bone marrow and host resistance parameters. Manuscript in preparation.
52. Boorman, G. A., Luster, M. I., Dean, J. H., and Wilson, R. E. The effect of adult exposure to diethylstilbestrol in the mouse on macrophage function and numbers. J. Reticuloendothel. Soc. 28: 547-560 (1980).
53. Dean, J. H., Luster, M. I., Boorman, G. A., Luebke, R. W., and Lauer, L. D. Effects of adult exposure to diethylstilbestrol on tumor susceptibility and host resistance parameters. J. Reticuloendothel. Soc. 28: 571-584 (1980).
54. Boorman, G. A., Luster, M. I., Dean, J. H., and Luebke, R. W. Effect of indomethacin on the bone marrow and immune system in the mouse. In preparation.
55. Luster, M. I., Dean, J. H., Boorman, G. A., Archer, D. L., Lauer, L. D., Lawson, L. D., Moore, J. A., and Wilson, R. E. The immunotoxicity of orthophenylphenol, Tris (2,3-dichloropropyl phosphate) and cyclophosphamide following subchronic exposure in mice. Toxicol. Appl. Pharmacol., in press.