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MUTAGENICITY OF MARINE POLLUTANTS AS IT COULD BE AFFECTING INSHORE AND OFFSHORE MARINE FISHERIES

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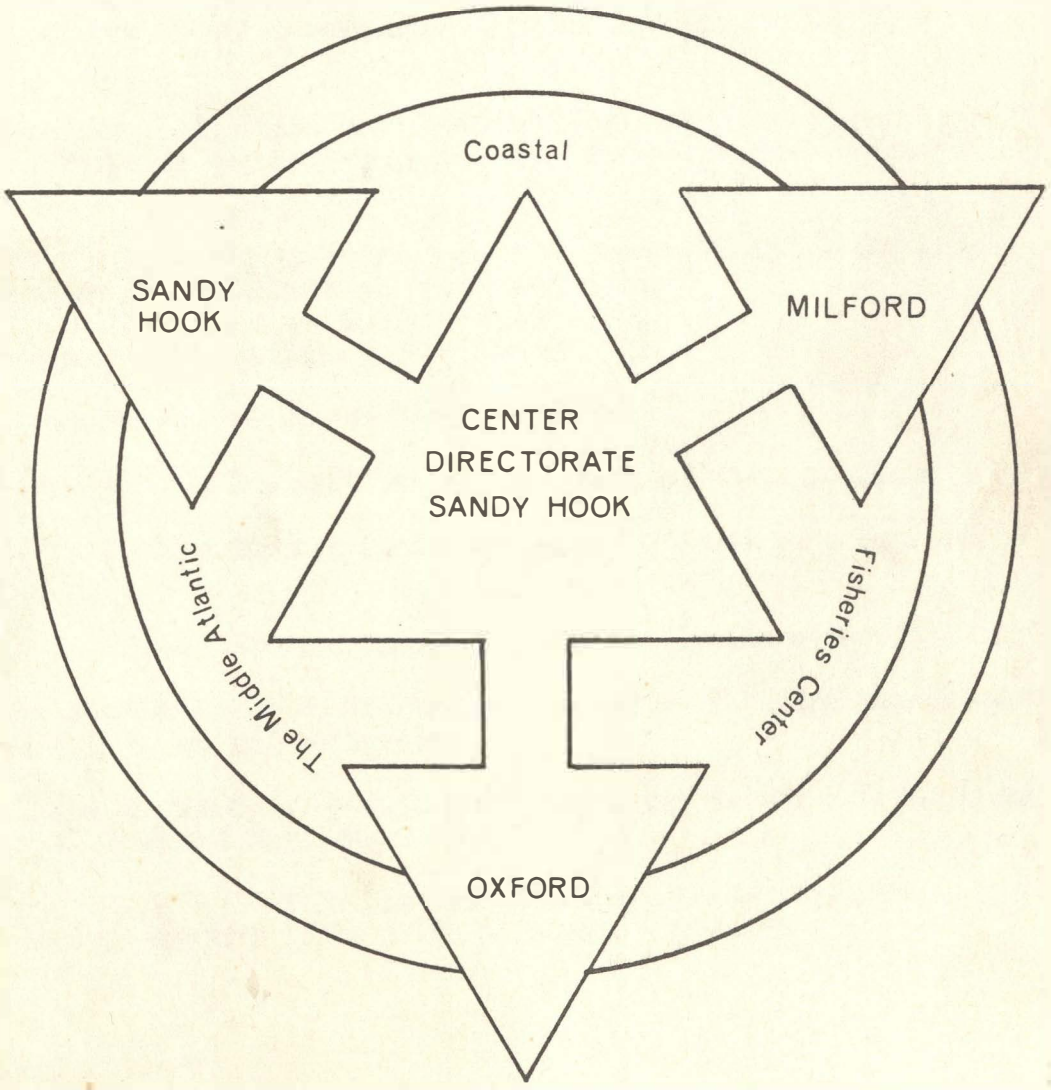
MIDDLE ATLANTIC COASTAL FISHERIES CENTER



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INFORMAL REPORT

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Marine Pollution, Short-term Acute, Long-term Chronic, and Genetic Effects

That modern industry has resulted in pollution problems of new dimensions with all living organisms constantly being exposed to a variety of contaminants - halogenated hydrocarbons, petroleum, toxic heavy metals, radionuclides, heat and nutrients - is commonly accepted knowledge. The escape into the atmosphere and entrance into the waterways of only one class of synthetic chemicals, the polychlorinated biphenyls, amounts to thousands of tons a year. The release of known toxic metals into the water and air has reached enormous magnitude.

Clearly, in many cases it will be deemed that there is no choice but to continue extensive polluting of the oceans. For example, the magnitude of oceanic oil pollution is likely to increase linearly with the worldwide growth of petroleum production, transportation and consumption. Even the most carefully controlled and monitored pollutant, radionuclides, will grow significantly over the next several decades. It is not only conceivable but probable that with increasing uses of atomic energy, accidents will occur that will result in damaging concentrations of radionuclides.

There appears to be no choice but to monitor the level and biological damage of pollutants. It has become increasingly evident that strictly man-oriented studies of pollution toxicity cannot serve the need for information on effects on natural

resources, not even in the field of radiation effects where there is such a great deal of information regarding man and higher animals. Although acute toxicity studies on marine forms have been conducted for several decades, little is known of the requirements of the most sensitive species, so results still do not indicate the "safe" radiation levels for the biota even for acute, short-term exposures. Research now though is more essential on the effects of low levels of chronic exposure to contaminants since lower-level, chronic exposure is the more widespread, "real-life" situation. Appraisal of just such long-term effects of chemicals in the environment is especially difficult. Although the long-range effects of continued exposure to some toxic materials are known for man, such data are very limited for other organisms - terrestrial or marine. Again, even for the most studied contaminant, radiation, it is generally acknowledged that information on the biological consequences of low-level chronic irradiation in the marine environment is still extremely limited.

Included in long-term effects of chronic, low-dose exposure to contaminants are genetic effects, particularly those resulting from those classes of marine contaminants that are mutagenic. Chemicals now in the environment may have either or both, individually or collectively powerful mutagenic effects. The diversity of mutagenic chemicals is so great there is no chemical reality in any group term which would comprise them all. Observed similarities in genetic action of these chemicals are due to limited possibilities of response whatever injury the genetic material has suffered. All populations, plant and animal, are now exposed to this variety of compounds encountered only in the last 30 or so years. Without prior exposure in evolutionary history it cannot be expected that genetic resistance could be developed on any significant scale in so short a time.

Genetic disturbances of the marine ecosystem must be so intimately interwoven with other non-genetic effects it is unlikely that the genetic parameters could be

totally excluded from measurement of non-genetic disturbances and vice versa. Certain aspects of the total genetic parameter though might be tested and considered alone. The results might then be used to monitor or appraise the effect of a contaminant or total contaminant load wrought largely through genetic interference.

Chemicals as Mutagens

In 1943, independent of each other, Auerbach in England and Oehkelers in Germany, proved that mutations can be induced in both plants and animals by chemical agents just as by radiation. Since then, the mutagenic activity of a great many chemicals has been tested on various organisms. As has often been the case with other cell reactions, the induction of mutagenic chromosome changes was at first thought to be specific for a very few substances. Since then, a great many new substances have turned out to have similar activity. These include nitrous acid and nitrites, bisulfites, peroxides, acridines, heavy metal salts and polynuclear aromatic hydrocarbons. In addition, some chemical compounds are metabolically converted to new compounds which can then react with the hereditary material of the chromosomes.

The mutagenic activity of alkylating agents, the largest group of chemicals interacting with DNA, has been proved in higher plants, fungi, bacteria, viruses, Drosophila, and other non-mammalian animals, like habrobracon and silkworms, cell cultures of mammals and man, and in human beings, as well as in experimental animals, in vivo. The pesticide dieldrin is an alkylating agent.

Various chemicals in combination can have either additive or synergistic effects. Further, there are for radiation-induced mutations a whole series of substances whose presence will reduce radiation damage. There are, as well, protective substances - chemical "promoters" - which will enhance the effectiveness of a chemical mutagen without themselves having any mutagenic effect.

In recent years the number of authors and publications describing mutagenic substances in the environment has added up to an impressive number. Yet there is a disquieting discrepancy, even in terms of the number of substances assimilated by the human body and the number adequately tested for mutagenicity.

Susceptibility of Marine Life to Environmental Mutagens

There can be little doubt but that fish, or indeed any marine species, are susceptible to deleterious genetic changes caused by mutagenic marine contaminants. The basic hereditary material of all life is the same. There is much general evidence for the mutagenicity of so many chemicals for such a wide spectrum of life - mammals, plants, and bacteria. There is further no evidence for species-specific carcinogens. The correlation between carcinogens and mutagens is close. Certainly, factors as assimilation and distribution of a substance in marine organisms and fish must be considered, as well as physiological activation of an otherwise non-mutagenic chemical or physiological de-activation of a mutagenic chemical. These factors though do not diminish the importance of the potential mutagen problem with respect to the oceans. Contrariwise it is accentuated considering the food chains of the marine environment typically of much greater length than those on land.

One characteristic of filter-feeding organisms, especially oysters, is their ability to concentrate a variety of compounds from the aqueous phase. In this way the contaminants may be accumulated to levels many times higher than the concentration in the medium. Such fat-soluble pesticides as DDT are, furthermore, concentrated as they pass from one feeding level up the food chain to the next. Genetically resistant species or individuals accumulating or tolerating high levels of a contaminant may aggravate the problem of accumulation in terminal trophic levels.

Pollution often does much of its damage by intensely hitting some one phase of the life cycle, usually some sensitive reproductive phase. The reproductive phase of commercial fishes is often completed in heavily contaminated estuaries. These heavily contaminated areas are the permanent residence, passage zone, or nursery area for about 80 per cent of all the commercially important fish and shellfish harvested in the United States. National pollution of these areas can have international effects on the fisheries when these areas are the nursery grounds for species fished by other countries.

Fish eggs and larvae, in addition, are more susceptible to toxic substances than are adults. Eggs spawned naked out into polluted waters where they must be fertilized and complete meiosis must certainly be highly vulnerable to genetic damage since fertilization and meiosis are genetically such very critical stages. In radiation genetics, the interval just before and just after fertilization has generally proved to be the most sensitive stage of egg.

Herring eggs have been demonstrated to accumulate cadmium rapidly the first few hours after fertilization with highest concentrations attained during this period. With the sea urchin, removal of metals from the seawater by formation of compounds with Versene contributes to an amelioration of developmental problems. Certain developmental problems can be characteristic of the culture of this species, such as slowness of development and malformation of the arms.

When the likelihood of additive and synergistic effects of various mutagenic marine pollutants is considered, the complexity of the problem in regard to commercial fish and the species on which they depend is realized. There is, in addition, evidence that factors as temperature and salinity influence the total damage accrued at least from radiation and heavy metals.

Nature and Consequences of Induced Genetic Change

A few combinations of mutations under certain conditions of exposure will have a particular selective value and hence be conserved by natural selection. Almost all mutations, however, are harmful if not simply lethal. As far as is known for all practical purposes, genetic damage is irreversible at the level of the individual. Chemical agents, as well as radiation, may produce genetic damage ranging from subvisible alterations of single genes to cell death. Microscopically visible chromosome breaks are one unit within this scale. Mutations can be either lethal or sublethal. They can be dominant or recessive or semi-dominant. Semi-dominant mutations can be expressed in either the heterozygous or homozygous condition, depending on other factors. Genetic damage can be done to the germ-line, to somatic cells, or to gametes. Most of the damage would be expressed as reduced fertility or fecundity; egg, zygote, embryo and larval death; and malformation. Also, to a large extent carcinogenesis and teratogenesis, and even to some extent degenerative cellular changes, all reflect changes in the basic genetic material. This is so since organ differentiation and functions are determined by genetic information. Chemicals which modify genetic material may so cause a variety of diseases of unknown etiology and developmental abnormalities. In the broad sense mutagenic effects are so of central importance.

Aside from the genetic effects of mutagenic environmental contaminants on the ecosystem, man's cultivated crops and domesticated animals risk adverse effects along with man. However, because breeding of most agricultural crops and animals is a well-controlled, quite artificial system, defective animals or crops strains can either be eliminated, or the mutagen eliminated from their environments. The overall economic loss need not be so great. (Reduction in any season's seed set for cereal crops intended for harvest rather than strain production could have more serious immediate effects.)

Deleterious genetic effects of a marine contaminant on a natural resource, where breeding occurs uncontrolled in the wild and the life history of the species is for all practical purposes uncontrollable, are an entirely different matter. Serious consideration of the consequences of induced genetic damage cannot but lead one to suppose such insidious changes could, in combination with other factors as overfishing, significantly reduce important commercial fish populations or an entire resource before the nature of the damage came to light. Even in humans it is possible for substantial environmentally-induced genetic damage to go forever undetected.

Aside from the scattered beginnings here and there of aquaculture industries, the commercial fisheries still depend on just such potentially vulnerable natural resources. Because a hatchery must carry its product through its most sensitive larval stage in smaller numbers than in the wild and in one spot, as opposed to many in the wild, water pollution can have now even a more disastrous effect in a hatchery than in nature. The Japanese regard water quality as the current most serious point to consider in establishing aqua-farms.

Lack of Information on the Mutagenicity of Marine Contaminants on Marine Life and Commercial Fish, and on the Consequences of Such for Commercial Fish Populations

Work being conducted on the general toxicity of marine contaminants, unfortunately, does not suffice for testing the mutagenicity of such contaminants. An animal or plant might exhibit no clinical symptoms of poisoning but yet show the cellular alterations that accompany the mutation process in the largest portion of cases. Even so there has hardly been any activity at all in respect to the mutagenicity of contaminants for marine species and commercial fish, equally so for the United States, Japan and Europe. No consideration has been given to the problem in either the national or international fisheries for either inshore or offshore species; nor has it yet been considered in connection with marine mammals.

This is at the same time there is an increasing demand for genetic evaluation of chemicals released into the environment, with suggestions that genetic tests become an integrated part of the more general toxicological protocol of testing. No doubt the failure of the marine and fishery fields to research the risk of contaminant mutagenicity along with other effects of toxic pollutants results, in part, from the very limited impact any aspect of genetic research has had to date on the fisheries or in marine biology. This is, however, bound to change and is, in fact, already changing with the advent of aquaculture and the still somewhat new field of ecological genetics.

Incorporation of Mutagenicity Tests into Standard Toxicological Protocol; Obtaining of Fixed Material for Cyto-genetic Study from Resource Assessment Cruises

As insinuated above, lethal sensitivity at the organismal level and mutation susceptibility tend to differ enormously from species to species. It is possible though to obtain highly relevant genetic information by using the animals from acute toxicity tests for cyto-genetic studies with regard to the production of chromosome aberrations in both somatic cells and germ cells. Information about germ-cell-stage specificity can be obtained from the same acute exposure animals using the dominant lethal test. Animals used in long-term feeding tests can also be used to obtain information on the genetic effects of chronic exposures. It is also possible to get important data on the occurrence of dominant lethal mutations in the germ cells.

Cyto-genetic study of mutagenicity can be field-oriented, as well as experimentally-oriented. Since cyto-genetic work depends largely on fixed samples, such should be obtainable in the course of cruises for Resource Assessment. This might often be accomplished without any additional sampling burden. Fixed fish tissues ought also to be obtainable by special arrangements with various fishing industries.

Genetics could supply the necessary new techniques of assessment more sensitive than those now employed to develop an unbiased measure of the effects of chronic low-dose exposure of commercial fish to contaminants that happen to be mutagenic, as well as toxic.

Probable Impact of Environmentally-induced Genetic Damage at Different Levels and for Different Aspects of Commercial Fish Populations

Population Response to Induced Genetic Damage

Lethal or sublethal dominant mutations will be rapidly eliminated from the population at the embryo or larval stages. Although dominant lethal mutations can have no effect from one generation to the next, they could be an important factor in the widely variable success of different years' spawn of commercial fish. This might be particularly so when chromosome level mutations are induced in the sensitive developing spawn.

Non-lethal mutations will tend to persist through several generations with the duration of their persistence being inversely proportional to the severity of their detrimental effects on vigor of the affected individuals.

If the mutations are largely recessive, they will be expressed only when in the homozygous state. The smaller the effective size of the gene pool, which is determined by the physical distance the population covers and the number of individuals in it, the greater the frequency of elimination by homozygosis of recessive lethal mutations. The mobility of all pelagic populations, and of a large fraction of near-shore populations as well, and the dispersal of the planktonic larvae of many benthic species insure a continual exchange of genetic material among separated populations. Consequently, there is a slow rate of elimination of lethal genes.

Open ocean and near-shore benthic organisms that do not produce pelagic larvae, and those planktonic species that undergo great seasonal fluctuations in abundance should eliminate more efficiently than pelagic groups environmentally-induced

recessive lethals from the population through death of homozygote carriers. This class of plankton appears to include a very large number of most important pelagic primary producers. However, there is not enough information to estimate minimum populations at any season. Also, there is not enough information to judge to what degree physical mixing processes may be able to produce new genetic intermixing during periods of rapid population increase. Hence any estimated rate of elimination of induced mutations would be most crude.

Deleterious mutations might be incorporated into the breeding system as useful components whereby the lethal combined with non-lethal gene results in a more vigorous individual-heterosis. The mobility of pelagic populations and the dispersal of planktonic larvae of benthic species would favor the development of such a system. Hybrid vigor produced by accumulated radiation-produced mutations has been shown to lead to significantly increased individual viability even up to levels of accumulated mutations that result in a considerable frequency of genetic death. Radiation-induced mutations in experimental caged mice may produce increased resistance to radiation, probably by heterosis.

In very heavily contaminated environments development of genetic resistance to a specific mutagen may be essential for survival of fish populations. In such cases the mutagen would no longer have an effect at levels that previously resulted in deleterious gene- and chromosome-level mutations. Fish populations in areas with a history of an insecticide contaminant may be resistant to the insecticide, and evidence suggests that resistance is genetic. Laboratory exposure of consecutive generations of mosquitofish to lethal levels of insecticides has yielded strains showing increased tolerances. In the marine environment resistance to a multiplicity of mutagens would have to be developed - perhaps at once - a situation for which there is no adequate experimental insight.

There are interesting indications of genetically-based tolerance to lead toxicity in a perennial grass, Festuca anima, growing on the top of an old Welsh lead line. Whether weedy plants initially very susceptible to certain herbicides develop resistance, is now being examined by a number of investigators in the plant field. It is well known that insects do develop resistance to insecticides. This capacity for developing genetic resistance, unfortunately, also makes for more environmental damage as heavier concentrations of the old pesticide or new ones are employed to control pests which have become genetically resistant.

Genetic recovery of a population from sustained sieges of environmental mutagens by whatever means could be expected to be a long-term process. Number of breeding generations per year would influence greatly the length of the recovery time which could take decades. Infertility, or more likely reduced fertility - genetic or otherwise - would be the limiting factor in the extent to which populations could overcome damage from a mutagenic marine contaminant. Before such recovery the affected population is subject to virtual elimination from any number of other factors from unusually heavy predation by another species, including exploitation by man, to damage by the harmful non-genetic effects of contaminants usually studied. Cyclic abundance patterns of many marine species make damage from a mutagenic pollutant especially serious when it is viewed as a form of predation.

Acceptable levels of recovery of a species, of populations, and of a fishery are entirely different though related matters. Exploited and often overfished commercial fisheries can hardly be expected to have the same rate of recovery as a part of the ecosystem unused by man. Because the fisheries are a commercial enterprise, as well as a natural resource, temporary reduction of a population through genetic damage of pollutants could mean the loss of the fishery or, more

often, economically significant portions of it. The potential for ultimate genetic recovery of populations would mean little to the immediate economics of the situation.

Of special significance in appraising genetic risk to a resource is the probability that some particularly critical species in the food chain would have an especially low sensitivity to a contaminant regarded as innocuous by tests on other species. It is generally recognized that differences among strains and species in response to chemical treatment have remained one of the major obstacles to estimates of genetic hazards of environmental substances. Marine species may be more closely linked than those of other ecosystems. If unique or very limited species interdependence is more common in the marine environment, then the tolerance of marine ecosystems will often be strongly limited by the most susceptible species.

Genetically-induced Sterility and Fecundity Effects

Genetic recovery of a population from the effects of mutagenic pollutants depends, as noted above, on the absence of significant contaminant-induced sterility. The very great fecundity of fish may seem to make any diminution of fertility by environmental mutagens of not so much significance. Yet the existence of commercial fish species and of entire ecosystems depends on the operation of just such a system of enormous fecundity.

Sterility is particularly pronounced for the mutagenic alkylating agents and for acute radiation. Radiation or chemical-induced sterility can, in fact, be so effective that it has been used to control insect populations through transfer of genetic material damaged by radiation or chemosterilants. Many of the chemosterilants are mutagenic and induce chromosome breakage. Treated insects pass on the lethal genes in an autocidal method of population control, commonly called the male-sterile technique. Apholate, one of those radiomimetic substances used

to produce partial or complete sterilization in mosquitoes, greatly reduces ovarian development and induces dominant lethal mutations in the eggs.

In insects egg production has been repeatedly observed to be depressed after treatment of female insects with a large number of chemicals, as well as by ultraviolet and ingested radionuclides. The number and the diversity of compounds that inhibit fecundity are extremely large. These include both effective and ineffective mutagens. Among these are chlorinated hydrocarbons, herbicides and arsenate. Data on the sterilizing effects of most chemical mutagens for higher plants and for mammals are regarded as inadequate. For marine fish data are yet less adequate.

Damage at the chromosome level is very often the basis for the several forms of sterility induced by different chemical agents. Gross chromosome abnormalities cause sterility in both males and females.

Oogenesis can be prevented by treatments that inhibit gonial cells from further dividing, resulting in their death. Egg production can also be inhibited by conditions affecting development in the nurse cells whose main function is to provide nourishment to the growing oocyte cell. This comes about when the nurse cell (the trophocyte) chromosomes are prevented from attaining the degree of ploidy which prepares them for the normal synthetic activity of oogenesis. This ploidy must be reached through repeated replication of the nurse cell chromosomes without accompanying division of the cell.

Environmentally-induced Dominant Lethal Mutations and Zygotic Death

Dominant lethal mutations are genetic aberrations that usually effect death of the zygote even though they are introduced by only one of the germ cells which unite at fertilization. They are generally eliminated in the first generation, usually even before or shortly after implantation in mammals. Dominant lethal mutations, therefore, do not contribute very much to the increasing genetic hazard

over the generations. They could cause, however, a marked reduction in any season's recruitment of new fish by reducing the number of successful zygotes.

All compounds inducing dominant lethals in mammals also induce other types of mutations in mammals, as well as in other organisms. For this reason, dominant lethal mutations are usually a good indicator of the overall mutagenic effects of a compound. They have been used extensively in genetic research, radiation biology, and in chemical mutagenesis.

At least in insect cells the induction of dominant lethal mutations usually does not hinder the maturation of the treated cell into a gamete, or the participation of the affected gamete in the formation of a zygote. A dominant lethal gene though does prevent the insect zygote from developing to maturity. Development usually ceases sometime prior to hatching. In some organisms, however, death is postponed to the larval or pupal stage.

Chromosome breakage in the sperm or egg nucleus accompanies dominant lethal mutation. This chromosome breakage leads to the production of chromosome imbalance in the cleavage divisions of the zygote. During cleavage divisions the formation of dicentric chromosomes and continued bridge formation result in uncompensated gene loss in nuclei which become progressively more unbalanced in their genetic content. Zygotic lethality comes about then through mitotic accidents and genetic imbalance in the developing embryos. Embryonic death is associated with a degression in the mitotic rate, and a complete cessation of mitosis may occur as early as the second or third cleavage division. Death of the embryo is often accompanied by polyploid cleavage nuclei, indicating that DNA syntheses may persist for some time after mitotic division ceases.

In fish dominant lethals might be induced in germ-line primordia or in developing germ cells. Mature unspawned germ cells would also be susceptible to their

induction by the portion of the fish's body load of contaminants finding its way into the mature gametes. Importantly these lethals though can, in addition, be induced in the spawned gametes. This is particularly so for the female gamete which is still in a phase of the genetically sensitive meiotic stage on being spawned. Once spawned the gametes have the stress of their cellular contaminant load compounded by that of whatever contaminants enter the gametes from the polluted waters in which they are spawned.

Environmentally-induced Genetic Damage to Spawned, Developing Eggs and Their Larvae

Genetic derangements of the chromosomes can most likely be induced in spawned developing eggs in the water column by mutagenic marine pollutants and even by simply cyto-toxic marine contaminants present in the water and taken up by the spawned eggs. They can also be induced at any point in development by the contaminant load the egg already had on spawning, particularly in the highly genetic sensitive post-fertilization period. Often these genetic disturbances will be on an order incompatible with further normal development of the eggs. Radiation experiments on the eggs of a wide variety of forms have shown that the earlier in development the genetic damage is done, the greater the effect.

There is considerable information about the degree of genetic disturbance at the chromosome level that can be tolerated by the developing eggs of different species when damage occurs at different stages of zygote development. Group tolerance of such aberrations depends on the level of organismic development and tissue complexity. A simple alga can tolerate more genetic damage than a mouse, which must undergo a complex embryogenesis. Zygotes or larvae with several cell points of induced genetic damage will develop into multiple mosaics for such damage. How serious the effect would be determined by the importance of the affected cell to embryogenesis or histogenesis, as well as by the extent of the genetic damage.

Tolerance for embryo-induced chromosome disturbances will also depend on whether the species is a diploid or a polyploid as is a good part of all plants. A tetraploid plant with four of each chromosome, instead of the usual two carried by diploid animals, might be expected to survive even with two of these chromosomes missing since there are two duplicates to supplement the loss. A mammal with two whole chromosomes missing would probably die as a blastula.

No doubt sensitivity of fish is far above that of even diploid plants. On the basis of radiation studies their sensitivity would be less, but would probably approach that of mammals. Their overall genetic sensitivity will be influenced by factors as rate of repair of damaged chromosomes, and how long an egg with severe cyto-toxic damage to the nuclear apparatus has to recover before it is too late for embryo development to resume. There are some indications that tetraploidy played a role in the distant evolution of present-day diploid fish.

Russian workers have reported reduction in vigor in fish cultures carrying low levels of radionuclide-induced chromosome damage that failed to kill or prevent normal development. There were, of course, other levels of damage that did kill and did prevent normal development.

Like any other plant or animal group, fish with minor chromosome abnormalities would likely fare better under conditions of artificial culture than in nature. It would seem that natural selection would eliminate most genetically defective fish from the wild commercial populations.

Environmentally-induced Genetic Damage to Somatic Tissues of Adults

Genetic damage to somatic cells is similar to that in germ cells. For both radiation and chemical mutagens breakage of chromosomes is a major cause of death. Chromosome breaks occur most rapidly in interphase cells preparing for division. The more proliferating tissue present, the more likely and the greater the degree of somatic damage.

The accumulated effect of somatic mutations can be detrimental to both life span and vigor. Somatic mutations have been linked to degenerative diseases in mammals. It is difficult to suppose that they are not a factor in fish pathology considering the extent of aquatic pollution.

In mammals somatic mutations have been linked also to malignancies and tumors. More than half of all agents mutagenic for bacteria are carcinogenic for animals. For mammals all the alkylating agents are carcinogenic, as well as mutagenic. It appears that all known carcinogens are also mutagens, but not all known mutagens are carcinogens. The connecting link between the mutagenicity and carcinogenicity of an agent is possibly a common capacity to break chromosomes.

Cyto-genetic Tests as a Means of Monitoring Mutagenicity of Marine Contaminants on Commercial Fish and Other Marine Species

Because of the specific localization and high DNA content of chromosomes, it is obvious that any change in the integrity of the chromosomes is reflected in DNA synthesis. In all mutation experiments it has been a fundamental experience that the appearance of artificially-induced gene mutations is normally accompanied by chromosome disturbances and vice versa. One study even showed a close correlation between the location of micronuclei, resulting from chromosome breakage of chromosomes misplaced at division, and mutation rates. There is also evidence for a correlation between chromosome damage and cyto-toxicity to the nuclear apparatus. Chemically-induced chromosome anomalies have so been used as a rapid indicator of overall potential mutagenicity, teratogenicity and carcinogenicity. Cyto-genetic tests are the prime means of determining mutagenicity at the mammalian level so essential for extrapolation of data to man.

Chromosome breaks and less specific cyto-genetic effects of mutagenic contaminants should be readily detectable in many commercial fish and other marine species. Already the considerable amount of cyto-genetic work done on shellfish

has shown this material to be desirable for such analyses. There is an advantage to using such material for mutagen testing over that of much of the higher plant and animal material because of the great fecundity of many of these marine organisms and the large size of their gonads. Eggs of aquatic species further present excellent material from the technical point of view since they can be directly exposed to the mutagen. The main difficulty with mutagenesis work in the classic genetic animal Drosophila concerns the distance between the point of exposure of the animals to the inducing agent and the point of final induction of the changes in the germ cells.

It is not meant to imply here that cyto-genetic-based tests could be the only tests for mutagenicity of marine contaminants in commercial fish or in the marine ecosystem. It is intended to emphasize, rather, the importance of a method which is relatively cheap and circumvents the necessity for artificial breeding or for much background information on the genetics of the organism. Even knowing the chromosome number of the species is not necessary. Furthermore, the method is directly applicable to the spawned eggs of important commercial fish - either experimentally spawned or field-collected. To develop a hatched fish a spawned egg must successfully complete the chromosome maneuvers of meiosis and cleavage and embryo mitoses, escaping embryo lethal effects of environmental mutagens throughout development. Further dominant lethal mutations carried by either gamete usually causing death sometime in embryo development are known to be associated with gross chromosome abnormalities.

Mutagenicity Analysis at the Cyto-genetic and Cytological Levels - Primary and Secondary Effects of Chemical Mutagens

As in radiation experiments, both primary and secondary effects can be easily distinguished in cytological action effects of chemical mutagens. With both

radiation and most of the mutagenic chemicals a mitosis-free period is manifested before the structural chromosome changes (mutations) typical of the secondary effects begin to appear.

Primary effects consist of pyknosis of all the mitotic phases. Pyknosis is one of the most common of toxic effects, and is also a leading feature among primary radiation effects. This manifests itself as chromosome stickiness with metaphase and anaphase chromosomes "melting" into strongly stained lumps. If anaphase ensues while the chromosomes are in this state, sticky bridges result which contain only chromosome matrix material as distinct from translocation bridges which also contain the chromonema of the chromosomes. However, even these sticky bridges may lead to true chromosome breakage with the appearance of free chromosome fragments in the cell. Stickiness of the chromosomes might also be responsible for errors of distribution, as well as mechanical breaks during the division process.

Resting nuclei under strong toxic or lethal conditions are contracted to small, strongly stained clumps, or they increase in size until they completely fill the cell. They can be directly fragmented into a large number of small chromosome pieces.

The secondary effects of chemical mutagens include c-mitosis, prophase poisoning and pre-prophase inhibition of mitosis, along with chromosome and chromatid aberrations of various types. Also, there can be mid-division of the chromosomes at anaphase so that the two resulting daughter cells have chromosomally unbalanced cell complements.

C-mitosis, one of these characteristic morphological pictures of physiological disturbances, is a reversible inactivation of the mechanism by which chromosomes move and are divided in cell division. This occurs along with excessive

chromosome contraction. Colchicine, a drug used to induce c-mitosis experimentally and in animal breeding, differs from chemical mutagens in that effective concentrations for inducing c-mitosis are below toxic ones.

Chromosome breakage can fall under the following categories: definite breakage; suspected chromatid breakage; chromosome gaps; multiple fragmentation of chromosomes; and chromosome pulverization. "Definite" breakage can be seen as the following types of configurations: translocation figures; acentric fragments; multicentrics; rings, centric or acentric; abnormal monocentric chromosomes as can arise by breakage and reunion of broken chromosome ends or by breakage alone.

There is often a continuous transition between the primary pyknotic-type effects of mutagens, and the secondary or mutagenic changes. Sometimes the pyknotic effects are separated from the secondary effects by a mitosis-free period. There are substances having secondary and mutagenic effects with very little or no preceding pyknosis. There are also substances with limited secondary effects but with a pronounced ability of inducing stickiness. Just as the primary effects can lead to specific chromosome lesions, so these latter can lead to mitotic disturbances.

Cells and Tissues of Commercial Fish Species and Marine Organisms Generally that Lend Themselves to Cyto-genetic Tests of Environmental Mutagens

Within any one species different tissues may vary in sensitivity. At the cellular level, the mutagenicity of chemical compounds, like the mutagenicity of radiation, depends largely on the kind of cytological and biochemical processes under way in the cells during the time of treatment. Somatic cells are not as sensitive to the induction of mutations as are germ cells. Even the various cell stages show considerable differences in sensitivity. Generally, more mature spermatocytes are more sensitive to ionizing radiation than less mature spermatocytes, spermatids and spermatozoa, which proceed with their regular development

and remain capable of fertilization after maturation. However, at least for initial work, which cells or tissues are used for studies on commercial fish and other marine species will be dictated largely by the practicality of the use and ready availability of the tissue for cyto-genetic study. This is particularly so for field-oriented work.

Somatic Tissue

In general, somatic tissue, aside from cultured peripheral blood of man and a very few select species, is not so easily studied for chromosome aberrations as is the gonadal tissue. Feasibility varies widely from species to species, and with respect to the experimental techniques available. There is no reason to believe that the somatic tissue of various marine species and commercial fish should, in general, present any more difficulties than experienced with mammals. This method might be particularly appropriate for the large marine algae, just as it has been particularly suitable for certain tissues of higher terrestrial plants.

Gill, scale and fin epithelium of commercial fish might regularly supply the dividing chromosome figures necessary for analysis of somatic damage. Developing tissues of late-stage larvae treated experimentally or field-collected once dissected out of the larvae could also be a reliable source of somatic mitoses from a sensitive phase of the life cycle of commercial fish.

Male and Female Gonads

Pre-meiotic mitotic divisions in the male and female gonads alike should supply a reasonably large number of chromosome figures on which to base estimates of cyto-genetic-level mutations. In most animals meiosis in the male and the preceding spermatogonial division are excellent study material. Such stages probably would prove equally good in commercial fish and marine crustaceans, including copepods from zooplankton. Female meiosis is prolonged and involved with yolk production. Even so, certain stages of the ripening and ripened unspawned eggs are suitable for cyto-genetic study.

Meiosis, Fertilization and Cleavage in Spawned Eggs

Meiosis in the female is not completed until fertilization. Hence, spawned marine eggs offer a unique opportunity for studying the latter portion of female meiosis, as well as the chromosomes contributed by the male gamete. Since the egg is a large cell, its chromosomes are generally quite suitable for study once interfering yolk or the blastodisc has been removed from the egg. Again, early cleavage divisions are very suitable for study because of the large cell size. Early cleavage stages appear from radiation studies to have increased genetic sensitivity over preceding stages of egg development. As embryo development proceeds and the cells become smaller in size, less detail about individual chromosomes and mitoses is observable, but cells are still usable for cyto-genetic study well on into larval development.

Indirect Determination of Chromosome Mutations in One-generation Breeding of Culturable Wild Species

Dominant Lethal Gene Test

As already noted above, dominant lethal genes are invariably associated with or, rather, are the expression of gross chromosome aberrations. All chemical compounds inducing dominant lethal mutations at least in mammals also induce other types of mutations as well in mammals and in other organisms. As many as 80 per cent of gene mutations in man are attributable to dominant autosomal traits. Dominant lethal mutations are so usually a good indicator for the mutagenic effects of a compound at least in mammals. They should be determinable in a one-generation breeding test of culturable marine species. This is providing mortality due to other causes can be controlled or quantitatively accounted for; also, that there is some cytological evidence for chromosome aberrations in a preliminary cyto-genetic study of the material intended for this subsequent genetic test. There is some evidence for induction of dominant lethals in fish by irradiation. Major

congenital malformations of the eye, head, back and tail defects in the rainbow trout, Salmo gairdnerii, following acute X-irradiation of mature eggs and sperm, may be associated predominately with gross chromosome changes.

Semi-sterility of F_1 Males and Chromosome Translocations

Translocated chromosomes, that is, the attachment of a broken chromosome to the end of another broken chromosome, lead to a portion of unbalanced gametes. This results in semi-sterility of F_1 males. When the number of dead embryos (or egg losses) is 50 per cent of the total number expected, semi-sterility of the male can be assumed. Semi-sterility itself is a method for detecting reciprocal translocations. For those commercial fish and other marine species whose early stage larvae, embryos or even zygotes could be handled in the laboratory or hatchery, such translocation-caused semi-sterility could be determined on the basis of zygote, embryo, or larval losses following experimental crosses.

Altered Sex Ratios

In some select culturable species losses of sex chromosomes could be used to appraise general rates of environmentally induced chromosome loss. Loss of sex chromosomes would be expressed in readily observable alterations in sex ratios, intersexes and hermaphrodites; also, in less directly observable alterations in fertility and fecundity.

Prevalence and Mutagenicity of Some Important Types of Marine Pollutants

Radiation

Of all the contaminants introduced into the environment radioactive materials are the most vigorously controlled, their effects longest studied and best understood. This stems from the fact that man is among the most radiosensitive organisms, and his greatest vulnerability lies in his genetic apparatus.

The main peaceful source of radioactive waste material arises during the processing of the spent nuclear fuel from reactors. The first significant release of radionuclides to the marine environment began in 1944 with the discharge of effluent from reactors at the Hanford atomic plant to the northeast Pacific Ocean via the Columbia River. Radionuclides are now found in all the oceans. Levels of radionuclides are regarded as still below the concentrations considered to be generally harmful to aquatic biota or man. Nonetheless, the possible expansion of power facilities requires further study of probable effects.

Most aquatic organisms are able to concentrate radioactivity from low-level radioactivity in waters, either by direct absorption or by the food they eat via the different food chains. Because mollusks ingest phytoplankton, detritus, and sediment, they are capable of greatly concentrating some radionuclides. One study showed that freshwater stream organisms had concentrated radioactive material to a considerable degree - up to nearly 1000 X that of the river water in some cases. This accumulation of radioactivity followed a definite pattern involving the different nutrition groups. Plankton and the filamentous algae, which absorb their nutrients directly from the water, had the greatest concentration. Herbivorous fish had a higher concentration than other species. The levels of radioactivity recorded for the plankton and algae were found to be directly related to the radioactivity level of the water.

The oceans are the world's oldest environment continuously available to living organisms. In the oceans marine species may have evolved under exceptionally low radiation levels. In such case there could have been natural selection against genes for radiation resistance. Marine species, consequently, may be more radiation-sensitive than better known terrestrial species. This is the view of Russia's Polikarpov (author of Radioecology of Aquatic Organisms, 1966).

Further, the ecosystems of the open oceans may themselves be intrinsically more sensitive to radiation, as to chemical mutagens, than many of the constituent species alone. The more closely interdependent the species, the more radiosensitive the ecosystem. Members of the marine ecosystem could well be more dependent on each other than species of land or freshwater ecosystems. The long, slow evolution of marine ecosystems, in response to environmental variations much more subtle than those that are critical on land or in freshwater, would have set the stage for development of such close dependence.

Values for LD_{50}'s of aquatic organisms show primitive forms to be more resistant than complex vertebrates, and older organisms more resistant than the young. Bacteria and algae may tolerate doses of thousands of roentgen, but freshwater fish are affected by considerably lower doses. The LD_{50}'s for rainbow trout ranged from 300 to 3000 R. Most of the freshwater and marine organisms for which data exist are relatively radioresistant though there are notable exceptions. Eggs and larvae are, unfortunately, more genetically sensitive. Surprisingly few LD_{50}'s have been determined for marine organisms, the majority of values determined for aquatic species being for freshwater organisms. The view has been expressed that these cultured marine species with determined LD_{50}'s may be only the "toughest", and that their radiosensitivity may not be representative of the others.

Apparent stimulation of growth by radiation in aquatic organisms is reported for periphyton, certain marine invertebrates, young blue crabs, and rainbow trout. In the case of periphyton there was a greater biomass and greater species diversity than in control aquaria. Other examples of possible stimulation of organisms by radiation under laboratory conditions can be found. Little or no knowledge exists though, either about the mechanisms involved or about the significance of radiation as a possible stimulus to individuals, populations, or ecosystems.

There was in Daphnia a continuous decrease of the intrinsic rate of natural increase as a non-linear function of the dose rate.

The reproductive capacity of mass cultures of Artemia was studied for several years. Strains descended from ancestors exposed to ^{32}P do not necessarily survive a second dose even though total dosage does not exceed the extinction dose given as an addition. A period of recovery involving generations must intervene. The reproductive potential of populations with different ancestral histories differs considerably. A test showed that maintenance of mass cultures at a particular level required only 0.2 per cent of the reproductive potential of the controls, but 1 per cent or more of the potential of the experimental stock.

Irradiation of chinook salmon at 0.5 R/day from the fertilization stage to the feeding stage produced no damage to the stock sufficient to reduce the reproductive capability over a period of slightly more than one generation. Although abnormalities in young fish were increased by irradiation, the number of adults returning was not affected. On the contrary, the irradiated stock returned in greater numbers, and produced a greater total of viable eggs than the control stock. The tetraploid evolution of salmon may be a factor in this response.

Also studied was the fecundity of a natural population of fish, Gambusia affinis affinis, that had been exposed to chronic irradiation in the same creek for many generations. A significantly larger brood size occurred in the irradiated than in a control non-irradiated population. However, more dead embryos and abnormalities were observed in the irradiated broods. This suggests that an increased fecundity is a means by which a natural population having a relatively short life cycle and producing a large number of progeny can adjust rapidly to an increased environmental stress caused by radiation.

Comparatively low-level chronic irradiation had a marked effect on continuing fecundity of the guppy.

After irradiation of plaice eggs no significant differences at hatching were observed in the survival or number of abnormal larvae produced (0.6 to 500 R total doses at rates of 10 mR/hr to 1 R/hr from fertilization to hatching). Russian workers (Polikarpov and Ivanov) reported on the effects of low-level radiation of a large number of marine and freshwater species on Black Sea fishes. Contrary to the general findings of other workers, their results indicate a greater sensitivity to chronic low levels of radiation. Experimental data concerning the radiation effects on developing embryos under laboratory conditions in radiation contaminated media are conflicting. Experiments have demonstrated the importance of the environmental factors on the radiosensitivity of aquatic organisms. It seems the effects of radiation on aquatic organisms can be evaluated only along with the effects of other major environmental factors. More studies should be established under rigorous, controlled experimental conditions. The effects of other environmental stress factors, such as salinity, temperature, oxygen and pollutants, must be studied and expanded to include the interaction of these factors with radiation effects.

Ivanov (1967) using more sensitive parameters reported on the effects of ^{90}Sr-^{90}Y in seawater on the mitotic activity and production of chromosome aberrations on the dividing cells of the Black Sea scorpion fish. As the concentration increased, the mitotic activity of the cells decreased. At the same time, the percentage of chromosome aberrations increased. The types of aberrations were varied, with chromosomal and chromatid bridges and fragments observed most frequently. At the highest concentrations abnormal mitoses were observed.

The salivary chromosomes of the larvae of Chironomous, which inhabit the contaminated bottom sediments of a creek at the Oak Ridge National (Atomic Energy) Laboratory, were analyzed for 5 years for aberrations. The absorbed doses of the

larvae were 1000 times the background. More than 130 generations had been exposed to this or greater doses over the previous 22 years. The conclusion of the study was that the ionizing radiation was increasing the frequency of new chromosome aberrations, but that these were being eliminated by natural selection.

In summary it may be concluded that radionuclide concentrations in the water acceptable to man must have subtle but difficult-to-measure effects on marine life. It is generally acknowledged that research on the biological and genetic consequences of low-level chronic irradiation in the marine environment is still extremely limited. What work has been done has been concerned mostly with somatic, not germ-line effects. In recent years the work on biological consequences of low-level irradiation has been expanded but it yet remains a subject of importance in all environments, including, of course, the marine.

More sensitive measures of effects on the biota are particularly essential for the low-level chronic exposures studied. This need for more sensitive parameters for measurement will no doubt increase the scope of the genetic work.

Many of the concepts that pertain to an understanding of radioactivity in the marine environment can also be applied to studies of other wastes discharged into the marine environment.

Heavy Metals

Heavy metals are listed among already identified contaminants of most concern. Metals have been dispersed into the environment as pesticides, as uncontrolled industrial wastes and emissions, and by other means.

Such metals numbering about two dozen are highly toxic to plants and animals, including man, in extremely low concentrations. The most toxic, persistent and abundant heavy metals in the environment include mercury, lead, arsenic, cadmium, chromium and nickel. They are biologically accumulated in the bodies of organisms,

remain for long periods of time, and function as accumulated poisons. They are concentrated in terrestrial and marine organisms from a few 100 to a few 1000 times the concentration in the surrounding medium. They may be synergistic, additive, or neutral to the toxic potential of each other.

As early as 1959 a German worker reported that salts of certain heavy metals induced chromosome breaks in plants. Before that, in 1945, a Swedish investigator reported an extensive study on the cytologic reactions induced in cells of root meristem by salt solutions of some 40 metals. Salts of heavy metals are one of three groups of substances now recognized as having a significant influence on chromosome breakage. Chromosome damage may occur at sub-toxic doses. The biochemical mechanisms by which heavy metals produce chromosome breakage though are not clear.

Aluminum, antimony, arsenic, cadmium, lead and tellurium salts have been reported to cause chromosome aberrations in such a wide spectrum of life as plants, insects, and cultured human cells. Some compounds of iron, manganese and mercury have all been reported to induce point mutations in microorganisms.

All of the metals cause a reduction in mitotic frequency, in many cases after first having stimulated division. They often have a rather high c-mitotic activity. In unphysiological concentrations they lead to a structural change of the chromosomes. Despiralization of the chromosomes in prophase is rather common and is especially marked after treatment with strong complex-forming metals. Fragmentation of the chromosomes is often observed. Chromosome stickiness can occur and lead to anaphase bridging. Changes in the stainability of the chromosomes indicate that the metals cause subtle chemical changes in them.

Genetic effects of heavy metal exposure are generally of the same type as those which occur from ionizing radiation and from treatment with some mutagenic

chemicals as mustard gas. Chromosome disturbances resulting from treatment with heavy metals show the same general morphology as those described for X-radiation, isotope radiation or treatment with mustard gas. The radiomimetic reactivity which is typical of complex-forming bivalent metals is, like ionizing radiation, also associated with gene-level mutagenic action. Work with plants on chlorophyll mutants has shown that all those kinds of mutants which appear spontaneously after ionizing radiation are also observed after treatment with metal ions.

As with X-rays and mustard gas, the most sensitive period of the cell cycle is early prophase.

With metal treatment the degree of reactivity though is less than with ionizing radiation. It has been observed that the optimal frequency of chromosome breaks appears somewhat later after treatment with metal ions than after treatment with ionizing and hard radiation. Generally chemical mutagens seem to induce predominantly chromatid-type aberrations of the chromosomes, whereas radiation treatment also induces chromosome-type aberrations (breaks in both chromatids of the chromosome).

Very few typical poisoning symptoms are seen after treatment with ionizing radiation. In contrast to this in the first stages after exposure to heavy metals there is a weak poisoning symptom with arrested mitoses and other reversibly disturbed narcotic cell effects. At some higher doses there is an irreversible inhibition or blocked or rearranged enzyme synthesis.

The concentration of free complex-forming metal ions needed for radiomimetic and mutagenic reaction is very low. The threshold value range at which an active metal gives visible chromosome breakage is normally narrow. With the heavy metals the first effect does not increase with increasing dose as after X-radiation, but

rather there is an optimum concentration. After treatment with stronger solutions a more or less total pyknosis of the cell is observed. When metals are added together the threshold value ranges remain as narrow as before and the number of disturbances is scarcely increased. Some heavy metals, when they are present in excess, can produce the phenomenon of inhibition, so that the presence of one ion disturbs the normal metabolism of another.

Varying the temperature will affect the outcome of results with the metals.

There is substantial evidence that certain metal ions are carcinogenic.

Below is given some information on the mutagenic, radiomimetic effects and general effects on reproduction of a few specific heavy metals, as determined from a variety of non-marine organisms. Because of the recent proliferation of literature on mutagenesis this brief summary by separate metal does not purport to be comprehensive. It is only intended here to give enough examples to convey the widespread nature of heavy metal mutagenicity.

Lead - Lead workers in Germany were examined for chromosome changes in leukocytes in peripheral blood. They showed a percentage of abnormal mitoses. The predominant chromosome aberrations were of the gap-and-break type. Most of the findings were statistically significant relative to control populations. Lead acetate added to normal human leukocyte cultures produced the same chromosome anomalies as found in these workers. Mice fed a diet containing lead acetate also exhibit an increased number of chromosome aberrations. Lead very efficiently leads to disturbances of the nuclear spindle which are of the kind that could lead to abnormalities of the chromosome number. Excessive fetal loss and malformed offspring resulting from lead intoxication have been reported for both experimental animals and humans.

Cyanide - The structure of plant nuclei is strongly affected by lethal KCn treatment. Resting nuclei in root tip meristem may lose their stainability and take on a spinous and distorted shape. When it is possible to stain such nuclei, chromatin is found dispersed into many fragments scattered all through the nucleus or pressed against the membrane. Chromosomes react to treatment with potassium cyanide by losing their matrix material and elongating. They become sticky. Anaphase may stop in a pyknotic condition. C-mitosis is also induced. At higher doses c-mitoses are influenced by the general toxicity of the treatments. At lower doses the c-mitoses take a more normal course.

Manganese - Manganese is mutagenic for bacteria and phages. The rates of chromosome aberrations induced by manganese are slight. They increase to a small extent with increasing concentration.

Aluminum - When applied to the floral spikes of barley and wheat $AlCl_3$ has a mutagenic effect. The same salt induces chromosome breaks in root tips of Vicia faba.

Arsenic - Inorganic arsenic exposure results in morphological manifestations of pyknotic changes in chromosomes, and arrested metaphases which do not involve spindle formation. The immediate effect of these changes is that cells are prevented from entering mitoses. Arsenic so causes a depression of proliferating tissue by mitotic arrest and chromosome pulverization. Salts of arsenic have been shown to induce chromosome breakage in cultured human cells. Long-term exposure to arsenical compounds has also led in man to similar chromosome breaks in leukocytes in vivo. It seems that some resistance to arsenic can be built up. Arsenic is a definite though slow-acting carcinogen for man.

Copper - Copper very efficiently leads to disturbances of the nuclear spindle which are of a kind that could lead to an aneuploidy.

Cadmium - Cadmium has a pronounced tendency to accumulate in the body. The biological half-time for total body in human is between 10 and 30 years. Yet, very little is known about the genetic and teratogenic effects of cadmium, the possible ameliorating effect of zinc, and the interaction of cadmium with other metals.

Studies have shown without doubt that cadmium and cadmium compounds can give rise to malignant tumors in rats at the site of the injection. Cadmium given as metal powder to rats induced tumors in which most of the metal powder was bound by the nuclei of the tumor cells.

Chromosomal aberrations have been induced by cadmium sulfide in cultured human leukocytes. There was also a higher-than-control frequency of chromosome breaks in the peripheral leukocytes of patients with Itai-itai disease. Cadmium nitrate caused chromosome breaks in root tip cells of Vicia faba.

Cadmium salts have been found to arrest mitosis and meiosis at metaphase by damaging the spindle.

Mercury - In 1937 it was reported that a fungicide containing ethyl mercury phosphate caused disturbances of mitosis and polyploidy in plant cells. A comparative analysis of the cytological effects on plant cells of several organic and inorganic mercury compounds has since been made. All mercury compounds studied cause c-mitosis, an inactivation of the spindle fiber mechanism at cell divisions similar to the well-known effect of colchicine. Mercury, however, often yields only incomplete c-mitoses with defective multipolar spindles and abnormal distributions of one or a few chromosomes. Abnormal distribution of chromosomes is almost invariably lethal at an early stage.

A cytological study of plant meiosis showed that methyl mercury also induced inactivation of the spindle fibers during meiosis resulting in chromosome effects corresponding to the ones induced at mitosis.

The dose at which methyl mercury interferes with chromosomal segregation is small. Apparently all mercury compounds are active as c-mitotic agents, although the effectiveness is considerably higher for organic than for inorganic ones. A genetic test for nondisjunction of chromosomes in Drosophila confirms the various cytological observations of the spindle-inhibiting effect of mercurials.

At least alkyl and phenyl mercury compounds also cause chromosome breakage. Data from one study tend to show a higher frequency of chromosome breakage in lymphocytes in humans being exposed to methyl mercury via fish. In rats a positive dominant lethal test has been observed after exposure to methyl mercury. This is indicative of chromosome breakage.

There is some evidence in plants that phenyl mercury induces somatic point-level mutations. Also, there is a clear tendency in Drosophila for an increased frequency of recessive lethals after mercury treatment. However, point-level mutations do not appear to be as serious an effect of mercury as do the chromosome-level disturbances.

In view of the stability of alkyl mercury compounds in the body the possibility of significant genetic effects must be borne in mind.

Pesticides

That certain pesticides - herbicides, insecticides, fungicides - may be classified as environmental mutagens has been amply demonstrated. Pesticides are a non-homogeneous group of chemicals whose cytological effects range from no observable effect to one greater than that for certain recognized mutagens.

Chlorinated hydrocarbons are the most stable class of pesticides, and may remain essentially unchanged in water and land environment for years. The

organic phosphate insecticides are believed to be less stable, some of them breaking down quickly in water. However, their partial decomposition may often yield substances of greater toxicity than the parent compounds.

The concentration and full effects of DDT in the open oceans are not known, there being no reliable estimate and no direct measure. However, marine fish are almost universally contaminated with DDT residues. Migratory fish, as tuna, carry up to 2 ppm in their gonads. Gray and sperm whales contain 0.4 and 6 ppm DDT in their blubbers. Other marine mammals can contain up to 800 ppm in their fat. Oysters can contain 0 to 5.4 ppm, there being highly variable local differences even in the same estuary. Estimates of fallout in rain suggest that one-quarter of the world's production of DDT may have entered the ocean. The amount in the marine biota itself is estimated to be on the order of less than 0.1 per cent of total production and it has already produced a demonstrable impact upon the marine environment.

The rate at which DDT degrades to harmless products in the marine system is unknown. For some of its degradation products, half-lives are certainly of the order of 5 years, perhaps even decades. If remaining DDT residues are in reservoirs which will in time transfer their content to the sea, we may expect, quite independent of future manufacturing practices, an increased level of these substances in marine organisms. If these compounds, in addition, degrade with half-lives of decades also, there may be no opportunity to redress the consequences. Signs of incipient damage to marine animals expected to occur with continuing accumulation of DDT have been reported.

In the speckled sea trout on the Texas coast DDT residues in the ripe eggs are about 8 ppm. This level compares with the residue of ppm in freshwater trout which cause 100 per cent failure in development of young fish. This can be regarded as presumptive evidence for similar reproductive failure in sea trout.

DDT concentrations in several species from the marine environment exceed those found to have deleterious effects in the laboratory. These concentrations have been correlated with population decreases or reproductive failures of a number of marine species.

Experimental evidence from copepods shows that the development of adults from nauplear stages is completely blocked when hatched from egg-bearing females maintained in seawater containing 10 pts per trillion DDT. There was significant mortality at 5 pts per trillion. These concentrations are lower than expected in rain water falling on the sea surface.

The fact that DDT can be accumulated to higher than whole body levels in the yolk material of the egg puts it in an excellent position to do genetic damage to the sensitive chromosomes of the developing egg or zygote.

Increased agricultural use of pesticides for insect, weed and disease control focused attention on the fact that certain agricultural chemicals may cause changes in the genetic constitution of organisms similar to those produced by radiation. Chlorinated hydrocarbons would, of course, be particularly hazardous because of their persistent nature. Pesticides, as well as other contaminants, may result in break-down products or metabolites more persistent, as well as more toxic, and possibly more mutagenic than was the original compound. If indeed pesticides and herbicides should under certain conditions alter or damage the hereditary constitution of a crop plant, their indiscriminate use would have significant agronomic effects on pure seed stocks of presumably stable genetic constitution.

There is now a considerable amount of experimentation directed at investigating the mutagenicity of pesticides. There is a 1971 book reviewing this subject for the pesticides already tested (authors Epstein and Legator). Several

pesticides have been found to have genetic effects similar to the highly mutagenic alkylating agents as ethyl methane sulfonate (EMS), the very much experimentally used and studied established mutagen from Eastman Organic Chemicals. In addition to its gene level effect, EMS induces a significant amount of cytological damage affecting mitotic index and chromosome integrity, and interfering with growth of the plant seedlings. The overwhelming majority of pesticides have not been adequately tested for mutagenicity although appropriate methodologies are available. It has been recommended in an official report (Report of the Advisory Panel on Mutagenicity of Pesticides to the Secretary - Commission on Pesticides and the Relationship to Environmental Health (HEW, GPO, December 1969) that no new pesticides should be registered until tested for mutagenicity. There are only about 400 chemicals commonly incorporated in current pesticide formulations.

Pesticides have now been observed to cause different types of chromosome aberrations - fragmentation, chromosome bridges, multiple anaphases and micronuclei. In meiotic cells extreme chromosome stickiness, fragmentation and bridges have been noted along with unequal and asynchronous division of chromosomes. Metaphase, anaphase and telophase fragments were the most common aberrations observed in two species studied. More of this damage from pesticides may be due to a severe physiological upset than is the case for X-rays and the chemical mutagen EMS.

Applications of 2,4-D to wheat and barley at any stage of growth produced various types of chromosome aberrations. Such results demonstrate that there is no developmental stage at which spraying may be done without endangering meiotic stability. Surprisingly, aberrations were found in pollen mother cells of plants sprayed as emerging seedlings. These aberrations could be caused by either

persistent transmission of abnormalities induced immediately on application of the herbicide or by residual persistent activity of the chemical. The latter appears to be the more probable explanation.

These 2,4-D induced cytological abnormalities are already known to be expressed in changes in awning, earliness (of flowering) and stature in wheat and barley. Dalijapan produced a wide spectrum of mutants in barley, wheat and oats. The mutant characters, as albino and dwarf, induced in the parental generation by some pesticides were transmitted to offspring at a low frequency.

Most pesticides were not designed with the aim of attacking the hereditary material. Exceptions to the rule, however, are chemosterilants designed specifically to produce dominant lethal mutations in insects giving rise to non-viable offspring. (There is evidence of active commercial interest in chemosterilants in Japan where extensive field tests are under way. Pollution by such chemicals would be extremely serious.) Some pesticides may act on DNA, but their specificity rests in details of penetration and metabolism in the pest. Most pesticides probably act as enzyme inhibitors. Their mutagenic actions may stem from incidental side effects of their own structure; from further metabolism in a species resulting in genetically active products; or from side effects of the inhibition of cellular enzyme.

Below is given some specific information on the particular mutagenic, radiomimetic and general effects of a few pesticides, as tested on plants, crops and mammals.

Metobromuron (-PAT) - Reduced seedling height, mitotic index, and germination percentage. Unlike EMS and radiation, induced primarily a severe physiological effect.

Atrazine - Induced numerical and structural chromosome abnormalities in plant microsporocytes during meiosis. Pollen mother cells with exact multiples of the basic chromosome number suggest that chromosomes might have continued to divide without cytokinesis. Sticky chromosomes indicate modifications of the surface structure of the chromosome. Micronuclei due to asynapsis and lagging chromosome pairs indicate some chromosomes were unstable during microsporogenesis and were excluded from daughter nuclei. Chromosome bridges occasionally found in anaphase and telophase indicate breakage which could lead to deficiencies and duplication of chromosomes. Chromosome fragments due to breaks were also observed.

Linuron - Induced chromosome breakage at low concentrations. Caused morphological changes in seedlings, inhibited root development and inhibited germination.

Monuron - Induced a high percentage of chromosome abnormalities. Caused physiological disturbances of cells.

Endrin - The most conspicuous changes in rat testes were seen in chromosomes. There were chromosome breaks, fragment chromosomes, ring chromosomes, chromatic bridges, and stickiness of the chromosomes.

Rotenone - Arrested chromosomes at metaphase.

Ethidium bromide - Caused progressive clumping of chromosomes at interphase.

DDT - Caused stickiness and liquification of chromosomes in cells of plant tissues.

DDVP - Induced chromosome gaps and breaks.

2,4-D (and related herbicides) - Similarity of cytological effects to those produced by X-rays was a warning that genetic changes may be induced. Application at any stage of plant growth produced various types of chromosome aberrations. There is no developmental stage at which spraying may be done without endangering

meiotic stability. At meiosis there are abnormalities that lead to production of monosomes, trisomes, and other aneuploides (all abnormalities of chromosome number), chromosome fragments and bridges: Mitoses are seriously affected. Other changes induced were presumed to be more gene-level effects.

Oil

Perhaps as much as 0.5 per cent of the world's total production of oil goes into the oceans. Although accidents cause the most evident damage to ocean resources, they make up less than 10 per cent of the estimated 2.1 million metric tons of oil introduced into the world's water. At least 90 per cent originates in day-to-day pollution. The magnitude of oceanic oil pollution is likely to increase linearly with the world-wide growth of petroleum production, transportation, and consumption.

There is only fragmentary information about the biological, let alone any genetic effects of this base load of oil in estuaries and coastal waters and on the high seas. Various dispersing agents, used to break up the oil into smaller drops and cause it to sink below the surface, are sometimes poisonous to ocean organisms. Even with a non-toxic dispersant, the dispersed oil is much more toxic to marine life than is an oil slick on the surface.

One possibly serious effect of oil dispersed over wide ocean areas could arise from the fact that mutagenic chlorinated hydrocarbons, such as DDT and dieldrin, are highly soluble in oil films. Among other things, studies should be made on the possible effects of different petroleum fractions on the reproduction of marine invertebrates and fishes in relation to probable mutagenic action of different fractions.

Summary

Chemicals introduced in the last 30 or so years and prevalent now throughout the terrestrial and aquatic environment may have either or both, individually or collectively powerful mutagenic, that is, hereditary altering effects, just as does radiation. The diversity of mutagenic chemicals is so great that no group term would comprise them all. A few combinations of mutations under certain conditions of exposure will have a particular selective value and hence be conserved by natural selection. Almost all mutations, however, are at least harmful.

Salts of heavy metals are one of three groups of substances now recognized as having a significant influence on chromosome breakage. Chromosome damage may occur at sub-toxic doses. That certain pesticides - herbicides, insecticides, fungicides - may be classified as environmental mutagens, has been amply demonstrated. Pesticides are a non-homogeneous group of chemicals whose effects on chromosomes range from no observable effect to one greater than that for well-known powerful mutagens experimentally used to alter genes. Such recognized environmental mutagens as heavy metals and pesticides, as well as many other chemicals, are also major marine pollutants.

The basic hereditary material of all life is the same. There is much general evidence for the mutagenicity of so many chemicals for a wide spectrum of life - mammals, plants, and bacteria. There is no basis for supposing that commercial fish and marine life generally are immune from the genetic effects of environmental mutagens, large portions of which find their way to the world's oceans.

Consideration of specific consequences of contaminant-induced genetic damage summarized in this report cannot but lead one to suppose that insidious genetic changes could, in combination with other factors as overfishing, significantly

reduce important commercial fish populations before the nature of the damage came to light. The biological potential for ultimate genetic recovery of these populations would mean little to the immediate economics of the situation. Consideration of the effect of mutagenic marine pollutants on spawn alone raises cause for concern. Fish gametes are spawned out into polluted waters where uptake of pollutants by the gametes is added to their already existing cell load of mutagens from the parent fish. Fertilization and completion of meiosis of fish eggs and early cleavage division of the embryo, which take place in polluted waters after spawning, are very genetically sensitive stages.

Most mutations are probably lethal, and, further, the expression of normal, unaltered genes and chromosomes is intimately interwoven into the very development, health, vigor, and reproductive performance of any organism. The extreme attitude or position then cannot be taken that the effects of environmental mutagens could be of no serious consequence to the commercial marine fisheries without concomitantly adopting the position that additional mortality and reduced reproductive potential would be of no matter to the fisheries. (Even should commercial fish withstand well the impact of mutagenic marine contaminants, the loading of fish with mutagenic chemicals and any metabolism of non-mutagens to mutagens in fish should eventually affect the general marketability of all fish.)

Work being conducted on the general toxicity of marine contaminants, unfortunately, does not suffice for testing the mutagenicity of such contaminants. An animal or plant might exhibit no clinical symptoms of poisoning but yet show the cellular alterations that accompany the mutation process in the largest portion of cases. Ways are indicated in this report that the mutagenicity of marine contaminants for commercial species might be researched as part of already

established toxicological protocols for fish. At the very least these studies would provide the new, more sensitive, much-needed parameter for appraising long-term, difficult-to-measure effects of chronic low-dose exposure to marine pollutants.

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