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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF PESTICIDES AND TOXIC SUBSTANC

DATE: JUL 21 1981

SUBJECT: Retraction of the Conclusion in My Previous Memorandum Stating that Pydrin® When Fed to Male Rats of the [CRL: COBS CD (SD) Br] Strain at 1000 ppm for a Period of Two Years Resulted in the Manifestation of Spindle Cell Sarcomas in the Subcutis Under the Conditions of the Test.

TOX Chem. No. 77A

FROM: Albin B. Kocialski, Ph.D. ABK
Section II, Toxicology Branch/HED (TS-769)

TO: F. D. R. Gee, PM #17
Registration Division (TS-767)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch/HED (TS-769)

This memorandum is issued as a retraction of my original conclusion that the administration of Pydrin® resulted in the manifestation of spindle cell sarcomas in male rats. This reversal of opinion is based upon the review of additional and more detailed information (previously not available) submitted by the Shell Chemical Company on June 15, 1981.

The original conclusion arrived at by this reviewer was based on the original data as it was presented. The Shell Chemical Company, when informed of the Agency's conclusion, did the following: (1) three board-certified veterinary pathologists on the Shell staff independently re-examined the original pathology slides and compared Dr. Hall's (contract pathologist) conclusions against their own findings and (2) they also resectioned and examined several of the tissues in question. A ten page addendum to the original study entitled "Addendum: Lifetime Feeding Study in Rats SD-43775 Technical LBI Project No. 20733-01" (attachment No. 1) was then submitted by the company contending that the five subcutaneous tumors in test males previously termed spindle cell sarcomas, actually represented at least three separate tumor types that were related only in their embryonal origin. Additionally, it was pointed out that three (or possibly four) of the fifty male control rats also showed malignant mesenchymal tumors or sarcomata one of which was a true spindle cell sarcoma. The Shell Chemical Company therefore concluded that what originally appeared to be a tumorogenic effect was in reality a spurious effect resulting from an unconventional tumor classification and assimilation.

The submitted ten page addendum was reviewed by the Toxicology Branch pathologist Dr. Louis Kasza. Dr. Kasza concluded (attachment No. 2) that the detailed description of the five malignant tumors in the high dose male group was sufficient to justify the new classification of these tumors. Furthermore, Dr. Kasza concluded that these neoplasms in the high dose male group were similar and comparable to three neoplasms in the control group.

This reviewer now concludes that the nearly equal incidences of sarcomata in the control male rats (3 or 4 out of 50) and the high dose male rats (5 out of 51) precludes the original interpretation of a generalized sarcomagenic effect.

The original conclusion is therefore retracted.

This memorandum also makes unnecessary any previously contemplated exposure and/or risk assessment for this chemical with regard to this issue.

Attachment

cc: Christine F. Chaisson
Caswell File No. 77A

OPP:HED:TOX: A.KOCIALSKI:sb 7/8/81 X77395 Rm. 824 CM 2 #1

Shell Development Company
A Division of Shell Oil Company

Toxicology Laboratory
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15 June 1981

ADDENDUM: "LIFETIME FEEDING STUDY IN RATS
SD-43775 TECHNICAL LBI PROJECT NO. 20733-01"

Shell scientists contend that feeding SD 43775 at 1000 ppm for two years did not elicit the growth of a unique subcutaneous tumor in males, termed "spindle cell sarcomas" by the contract laboratory pathologist. It is felt the five tumors so diagnosed actually represent at least three separate tumor types, related only in their embryonal origin.

The term "spindle cell sarcoma" denotes a malignant tumor of mesenchymal origin which contains cells with a "spindled" or fusiform morphological appearance. Since such a diagnosis addresses only the histomorphological features of the tumor, it is generally restricted to those tumors for which the site of origin cannot be definitively determined or lack areas of cellular differentiation; i.e., fibroblasts, chondroblasts, osteoblasts. Most sarcomas are classically described according to such areas of differention, prefaced by modifiers defining the degree of differentiation, i.e., poorly-differentiated fibrosarcoma. Identification of such areas of cellular maturation may require multiple sections throughout the tumor mass or the aid of specialized histochemical methods.

Independent histological evaluation of the five tumors in question by three board-certified veterinary pathologists on the Shell staff consistently confirmed the morphological heterogeneity of the tumors. Formalin-preserved tumor tissue was obtained from the tissue archives and multiple additional sections were processed for microscopic evaluation. Special stains were not employed due to the timeliness of the 11 June 1981 meeting. These additional

sections further substantiated the initial contention that the tumors were morphologically dissimilar and should not be considered a single entity.

Rat number 7227 was found dead on 23 November 1977 with a 4 x 3 cm subcutaneous, ulcerated mass in its right axillary region. The comment advanced by the pathologist following his diagnosis of spindle cell sarcoma was:

> "the mass is composed of highly cellular nodules of mesenchymal cells streaming and whorling in the subcutis. The lesion is highly infiltrative and composed of cells with plump but elongated nuclei often polymorphic in appearance and containing finely stippled chromatin with an occasional small nucleolus. Nuclei were very densely compacted in some areas and in others are separated by dense fibrous connective tissue. In some places the connective tissue appears pre-existant because of the presence of small, mature, non-malignant fibrocytes. Mitoses are frequent in some areas of the lesion. A storiform pattern is found in many areas of the lesion"

All Shell pathologists contend the development of "dense fibrous connective tissue" was not merely a desmoplastic reaction or a pre-existing anatomic structure, but represents a portion of the proliferative lesion. Therefore, a diagnosis of poorly-differentiated <u>fibrosarcoma</u> is more valid under current medical mores.

Rat number 7188 was sacrificed in moribund condition on 18 November 1978. During necropsy, a large (14.6 x 10.3 x 3.9 cm) mass was removed from the subcutaneous tissues of the ventral cervical region. Its outer surface was white and its cut surface was gray and grainy. An early area of cutaneous ulceration was described. Microscopically, the mass was described as:

> "the spindle cell sarcoma is composed of large pleomorphic cells with a spindle cell pattern in most areas and fibrous connective tissue formation. Many areas of necrosis are found. Cells are large, bizarre, highly anaplastic and tend to whorl around blood vessels. Mitotic figures are numerous with atypia of many. The lesion in some areas appears like it is making osteoid and in other areas as it is making fibrous connective tissue. Mammary gland ducts are found in one portion of the lesion"

Returning to the cut tissue, it was obvious a multilobular structure was present in the mass. Sections prepared from the tumor further clarified the relationship of the tumor to the mammary gland. A large mammary fibroadenoma, a common tumor of the laboratory rat, comprised the center of the mass. At its periphery, more bizarre, anaplastic cells were present which resembled the tumor described above. Larger areas of osteoid deposition and resultant mineralization were seen in these new sections. Additional areas of loose, areolar connective tissue, composed principly of stellate cells, were observed. These patterns suggest a diagnosis of malignant mixed mammary tumor is more appropriate.

Rat number 7213 was likewise sacrificed in a moribund condition on 11 October 1978. His left hindlimb contained a 3.5 x 3.0 x 2.0 cm ulcerated mass. A diagnosis of spindle cell sarcoma preceeded the following histomorphological description:

> "the hind limb mass is composed of proliferation of mesenchymal cells in multiple highly cellular nodules, each cell surrounded by abundant fibrous connective tissue; although not prominent, a storiform pattern is found in the lesion; individual cells are large with large nuclei; mitotic figures were abundant in some areas of the neoplasm; the lesion is highly infiltrative; epidermis is ulcerated"

In the opinion of the Shell pathologists, sufficient differentiation of fibroblasts and collagen deposition were present to classify this tumor as a fibrosarcoma.

Rat number 7224 was sacrificed at the termination of the study on 5 January 1979. The left lateral thorax contained a firm, scab-covered, white, lobular, 7 x 3 cm, exophytic mass with a narrow base and no subcutaneous growth (keloid). Again, a spindle cell sarcoma was diagnosed based upon the observation that:

> "the spindle cell sarcoma is composed of mesenchymal cells forming bundles of fibrous structures, whorls, and in some places, blending in with nerve sheaths. It is most likely of nerve sheath origin or histiocytic"

Although areas of pallisading cells suggested a peripheral neural origin, additional sections were not supportive. Sections through the stalk of the polypoid or exophytic structure revealed the dense collagenous nature of the tumor. A unique histological feature was a marked proliferation of pericytes surrounding small vessels entering and within the tumor. This perivascular proliferation formed concentric lamellae with thin fibrous strands interspersed, producing a thightly cuffed or honeycombed appearance. Such anatomic features are generally observed in tumors arising from pericytes or hemangiopericytomas. However, since the general pattern of the tumor is more suggestive of a fibrosarcoma, this reaction may be secondary. It may also account for some of the pallisading appearance which led the contract laboratory pathologist to the consideration of a nerve sheath tumor.

In rat number 7230, a tan, firm, 1.5 x 1.0 x 1.5 cm nodule was attached to the subcutaneous aspect of the skin in the Left perianal area when presented for terminal necropsy on 5 January 1979. Although the contract laboratory pathologist included this tumor in the group of spindle cell sarcomas, his description is incongruous with this proposed diagnosis, yet is consistent with our diagnosis of liposarcoma:

> "the sarcoma in the perianal area is composed of nodules of tumor cells surrounded by fibrous connective tissue. The cells in the nodular areas tend to interweave and whorl. Numerous variably-sized round vacuoles are found within cells, the latter which appear like lipoblasts. Multinucleated giant cells are also seen. The lesion probably represents a liposarcoma or xanthomatous change to a histiocytic tumor. In the prefemoral lymph node, there is a focus of cells composed of large nuclei and a slight appearance of their being attached to one another. The lesion is equivocal for being a metastasis infiltration into the node"

Additional sections have not been evaluated from this tumor, yet it is obvious from the above description and the representative slide that this tumor lacks cells histomorphologically defined as "spindled" and thus, cannot be classified as a "spindle cell sarcoma". Its only relationship to such a diagnosis is that it is a sarcoma. Cells were polyhedral to spherical, not fusiform. They arose from the dermis adjacent to sebaceous adnexa. The vacuolation suggested poorly formed fat droplets and supported the diagnosis of liposarcoma. Although a negative fat stain (oil red O) of the tumor was present in the archives, the section profile was identical to that stained with H&E suggesting tissue previously processed with fat solvents was used for this histochemical technique. Therefore, a negative stain is inconclusive. Additional tissue is being sought to repeat the fat differentiation, but the small size of the tumor may have resulted in the processing and embeddment of all available tumor tissue.

From the above discussion, it should be apparent that the so-called "spindle cell sarcomas" were not unique tumors, but common only in their embryomal precursor cell. Thus, what appeared to be a tumorogenic effect was only the result of unconventional tumor classification and assimilation.

Four of 50 male control rats also contained malignant mesenchymal tumors or sarcomata (see attached table). A "spindle cell sarcoma" was diagnosed in a single control male rat. The tumor was described as a firm, white, fatty mass measuring 5 x 5 x 3 cm adhering to the sternum and diaphragm. It occurred in a rat sacrificed in moribund condition on 1 May 1978. Recent examination of

the preserved mass revealed it to be confined to the right posterio-dorsal aspect of the thorax and actively invading but not penetrating the overlying intercostal muscles. It was described by the contract laboratory pathologist:

> "Intrathoracic mass is a highly cellular mass of tissue with the cells forming long bundles which interlace and whorl. Individual cells are large with large oval plump nuclei, marked atypia and numerous mitotic figures. Areas of necrosis are found, and there is vascular invasion. The origin of the lesion is not known."

The tissue was resectioned in various areas, including the tumor subjacent to the intercostal muscles. The character of the lesion remained constant or homogeneous as described above. Adipocytes were scattered throughout the mass, but no definitive evidence of adipocytic differentiation was present in sites of cellular interface. Therefore, the origin of the tumor was not ascertained and the term "spindle cell sarcoma" is deemed appropriate.

Three additional male rats in the control group were identified as containing sarcomata, including a widely dissiminated osteosarcoma, a duodenal hemangiosarcoma and an ileal leiomyosarcoma.

Rat number 7105 was euthanized in moribund condition on 27 December 1978. Nodules were observed in the liver and lungs during necropsy. Microscopic evaluation of the tissues by the contract laboratory pathologist led to the identification of two systemic disorders. Lymphocytic leukemia infiltrated vessels and tissues of the liver, brain, kidneys, lungs, spleen, heart and gastrointestinal tract. Metastatic foci of osteosarcoma were identified in the lung, liver, kidney and myocardium. The primary osteosarcoma was not found. Lesions were characterized as:

> "Highly cellular, irregular, round nodules composed of spindloid or polygonal cells secreting a matrix of eosinophilic osteoid which is mineralized. Mitotic figures are frequent."

These lesions have been confirmed by a Shell pathologist.

Rat number 7127 was likewise euthanized in moribund condition on 19 December 1978. A small (1/2 cm), round, dark red mass was attached to the outside of the duodenum and two masses were isolated in the subcutis of the right side and left axilla. The latter two lesions were diagnosed as benign tumors of mesenchymal origin (fibroma and lipoma). The tumor arising from the serosal surface of the duodenum was not described, but diagnosed as an hemangiosarcoma. Review of the slide by a Shell pathologist confirmed the earlier diagnosis. The lesion was a polypoid, uncircumscribed mass apparently arising from the site of mesenteric attachment to the duodenal serosa. The outer, longitudinal muscle layer of the duodenum was focally, slightly hypertrophic at the junction with the tumor. A large vein communicated through the stalk, connecting submucosal and extraserosal tissues. The tumor was principly formed by fusiform or spindled cells separated by slit-like, poorly-formed cavities, often containing variable numbers of erythrocytes. Both cystic areas and areas of densely-packed cells were scattered throughout the mass, yet the predominant pattern was loose or areolar. In some areas, cells became more stellate to polyhedral and individualized. Nuclei were elliptical to polyhedral, moderately basophilic, outlined by a distinct nucleolemma and contained a finely granular chromatin and a pale nucleolus. Mitoses were not seen. Cytoplasm was scant, lightly eosinophilic and filamentous. No metastases were present in sections of mesenteric lymph nodes available for evaluation.

An ileal leiomyosarcoma was diagnosed from sections from control rat number 7134. The 1.5 x 2.0 x 2.5 cm, fluxulant, mottled gray and red mass was discovered attached to the proximal ileum, 30 cm proximal to the cecum, during the terminal necropsy on 5 January 1979. The histomorphological description

was not included in the report from the contract laboratory. The mass was evaluated by all Shell pathologists and was found to arise from the muscularis externa of the ileum. The mass was histologically uncircumscribed, polypoid, well-delineated and cystic. Muscle bundles were in disarray between the mass and the ileal mucosa. Multiple, isolated, cystic, metaplastic glands were interpersed in the partially-muscular tumor stalk, and the large cystic cavity within the mass was incompletely lined by similar, simple columnar to cuboidal epithelium. The cavity contained numerous polymorphonuclear leukocytes, necrotic debris, dystrophic mineralization, proliferating fibroblasts, lipophages and cholesterol clefts from dissolved cholesterol crystals. Underlying the surrounding epithelium was a thin supporting stroma. Near the stalk, a zone of proliferating, polyhedral cells with unstained to lightly eosinohilic, spherical cytoplasm with distinct cell membranes and spherical to elongated, moderately basophilic nuclei. Nuclei lacked nucleoli, distinct nuclear membranes and mitotic activity. These cells formed a solid sheet with negligable fibrovascular stroma. Similar cells extended into the base of the tumor, infiltrating between differentiated muscle bundles and isolated glands. The distinctive cell membranes, mature and spherical to polyhedral nuclei in these highly cellular areas suggested a hyperplastic, proliferative response, as opposed to neoplasia. Yet, foci of mitotic activity and atypia alluded to neoplasia. Whether this lesion is a profound, local, chronic inflammatory response or a localized sarcoma requires additional sections and specialized staining procedures, which have yet to be performed.

Regardless of whether the ileal lesion in control rat 7134 is truly a sarcoma, the nearly equal incidence of sarcomata in the male control rats (3 or 4/50) and the male high-dose rats (5/51), precludes the interpretation of a generalized sarcomogenic effect arising from the chronic exposure to 1000 ppm of SD 4.3775.

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15 June 1981

Group Male Controls
Dose 0 ppm
Study 20733-01

Animal No./Tissue		Diagnosis: Litton	Diagnosis: Shell
7134	Ileum	Leiomyosarcoma	Histiocytic sarcoma or chronic inflammation
7105	Liver/Lung	Osteosarcoma	Osteosarcoma
7110	Thorax	Spindle cell sarcoma	Spindle cell sarcoma
7127	Intestine plus subcutis (x2)	Hemangiosarcoma plus fibroma plus lipoma	Hemangiosarcoma, fibroma, lipoma
7107	Subcutis	Fibroma	Fibroma
7097	Subcutis	Fibroma	Fibroma
7087	Mammary	Fibroadenoma	Fibroadenoma

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15 June 1981

Group 1000 ppm Males
Dose 1000 ppm
Study 20733-01

Animal No./Tissue		Diagnosis: Litton	Diagnosis: Shell
7188	Mammary	Spindle cell sarcoma (osteoid)	Malignant mixed mammary tumor
7213	Subcutis	Spindle cell sarcoma (osteoid)	Fibrosarcoma
7224	Subcutis	Spindle cell sarcoma (neuro)	Fibrosarcoma
7227	Subcutis	Spindle cell sarcoma (neuro)	Fibrosarcoma
7230	Perianal Skin	Spindle cell sarcoma (liposarcoma)	Liposarcoma
7189	Subcutis	Neurofibroma	Neurofibroma

CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460
June 26, 1981

OFFICE OF TOXIC SUBSTANCES

MEMORANDUM

TO: Albin Kocialsky, Ph.D.
Toxicology Branch, TS-769

FROM: Louis Kasza, D.V.M., Ph.D. L. K
Toxicology Branch, TS-769

SUBJECT: Pathologic Evaluation of Addendum, Shell Pydrin Report

In the Addendum, the detailed description of the five malignant tumors in the high dose group justified the new classification of the five sarcomas. Also the detailed description of the three malignant tumors in the control group indicates that the diagnosed neoplasms in the high dose group (5 sarcomas) and in the control group (3 sarcomas) are comparable.

In the evaluation of the significance of the incidence of sarcomas in the high dose group, the above-mentioned findings should be considered.