a residue was even found in muscle tissues), with implants smaller than the size permitted, 120 days after implantation (and also shorter periods after implantation), the results reported show that DES residues will appear at low levels in the edible tissues of cattle implanted in accordance with approved conditions of use. The fact that part of the DES implants still existed in some steers at the end of 120 days (and were thus presumably sending DES into those animals' systems at the day of slaughter) buttresses this conclusion.

(c) *Oral Dosages in Sheep.*—(i) *Study.* DES is permitted in sheep feed at up to 2 mg per head per day, again with a 7-day withdrawal period, 21 CFR 558.225. One study was done with sheep by Dr. Aschbacher (G–4). In this study, 6 sheep were sacrificed 7 days after feeding with a single dose of ^{14}C-DES. Neither the report nor the testimony is clear as to the amount of the ^{14}C-DES dose. Each of the sheep had been fed DES for the 7 days prior to the C-DES feeding, 2 at the rate of 100 mg per day, 2 at 4 mg per day, and 2 at 3 mg per day. DES feeding was continued in the first two groups for an additional 7 days but was stopped after the date upon which the ^{14}C-DES was fed in the third (3 mg) group. All sheep were sacrificed on day 15 (7 days following ^{14}C-DES dosing).

No measurable radioactivity was observed in the tissues of any of these sheep, with the exception of the adrenal glands in three sheep. In his testimony, Dr. Aschbacher stated that the design of this experiment and its analytical procedures would have prevented the quantitation of radioactivity present at the level of less than 1 ppb of DES equivalents in the animals receiving 4 or 3 mg of unlabeled DES per day (G–1 at 3). (He did not address the sensitivity of his methods as they apply to animals receiving 100 mg per day. The lack of sensitivity would also, however, mean that residues below 1 ppb from those animals would not be detected.)

(ii) *Conclusion As to Oral Dosages in Sheep.* The most likely reason for the failure of this study to show residues in tissues other than the adrenal glands is the relatively high limits of detection of the test methods. (Many of the residues observed in the cattle studies were observed at levels below the lowest level of sensitivity (1 ppb) of this test.)

The presence of DES residues in the adrenal glands of the sheep tested is evidence that DES residues remain within the sheep's bodies rather than passing totally out of their system. Because no rationale has been advanced to support a theory that all DES residues in the sheep's body would be concentrated in adrenal glands, I must conclude that DES residues would be present, at non-observable levels, in the other tissues of sheep fed DES.

My conclusion on this subject is supported by the results observed in the cattle studies discussed above. The fact that both cattle and sheep respond to DES by increased growth warrants, for present purposes, the assumption that the two animals deal with ingested DES in a similar manner. Such an assumption is biologically plausible (and more likely than the contrary assumption), and nothing in the record contradicts it. Both cattle and sheep are reminants and are good models for ruminant metabolism.

I find that the results of the radiotracer study in sheep, taken together with the evidence from the cattle studies, show that DES used under approved (or actual) conditions of use results in DES residues in edible tissues of treated sheep.

(d) *Implants in Sheep.* No radiotracer study was performed with implanted sheep. Although the question is not without difficulty, I conclude that the conservative approach appropriate for safety determinations sanctions extrapolation from the cattle data, despite species differences, to determine that DES implants in sheep result in DES residues in the edible tissues of sheep. I have discussed above my reasons for concluding that sheep are likely to deal with orally administered DES in a manner similar to cattle. The same considerations apply to DES implants. The results of the radioisotope test of DES fed to sheep (which showed that fed DES does remain in at least some tissue of these animals) also lend some support to the conclusion that DES implantation in sheep leads to tissues residues. I therefore find that the radiotracer studies show that use of DES implants in sheep in accordance with approved conditions of use results in DES residues in edible tissues of the treated animals.

(e) *The Pseudo-DES Issue.* The Court ordered a hearing on the withdrawal of approval of the DES NADA's in part due to applicants' argument that the residues identified by the radioisotope procedure were caused by an impurity in the DES implants supplied to the government researchers by Hess & Clark, *Hess & Clark, Division of Rhodia, Inc.*, v. *FDA, supra,* 495 F. 2d at 992. In particular, the applicants argued that the implants were contaminated with "pseudo-DES," which is somewhat similar in chemical structure to actual DES.

The Bureaus argue that Hess & Clark withheld the information that there were impurities in the implants until the radioisotope studies were completed and revealed that information only when it became in Hess & Clark's interest to do so (Bureaus' Brief at 62; see also the cross-examination of Dr. Tennent (Tr. at 1274–76)). The manufacturers, on the other hand, argue that they had forewarned the agency that there were impurities in the implants (Tr. at 1275). I need not decide this issue.

To resolve the pseudo-DES question, Dr. Williams further tested the liver samples from Dr. Rumsey's steer studies. These tests showed that the radioactivity identified by him as DES or its conjugates was, in fact, authentic DES or its conjugates and not the impurity, pseudo-DES (G–99 at 3–5; G–101; G–102).

Two of the manufacturers' witnesses discussed the pseudo-DES problem. One of them, Dr. Lieberman, admitted on cross-examination that in light of Dr. Williams' work all of the observed residue could not be pseudo-DES (Tr. at 2116).

The manufacturing parties' Dr. Tennent presented, in his direct testimony, calculations he had made from Dr. Williams' results. He stated that he had made corrections for contamination. He found 0.035 ppb of DES and its conjugates in the 120-day steer implanted with 1 implant and 0.120 ppb in the 120-day steer implanted with 2 implants (M–132 at 15). A table made up by Dr. Tennent for the samples taken from the orally dosed animals showed 0.037 ppb DES and conjugates in one animal slaughtered after a 7-day withdrawal period and 0.011 ppb in the other animal with the same withdrawal period (id. at 17). Thus, Dr. Tennent's analysis seems not to dispute the fact that there was some actual DES and/or its conjugates in the livers of some of these animals. Although Dr. Tennent stated that he considered the data marginal, due to inherent counting errors at low levels of activity (M–132 at 16), the record shows that Dr. Williams minimized counting errors by extending the counting time in his test procedure (Tr. at 684).

I find that the residues detected cannot be attributed wholly to pseudo-DES or other impurities. Whether or not, as the manufacturing parties' witnesses contend, some of the residues detected as DES and/or its conjugates are impurities, it is clear that part of the observed residues are in fact DES residues. I cannot find, for the reasons discussed below in the section of this Decision (sections III(C) and (D)) dealing with the carcinogenicity and other adverse effects of DES and its conjugates, that any amount of DES residues is safe. Therefore, the fact that DES residues have been shown to occur

at low levels in the edible tissues of DES treated animals (together with the evidence on toxicity discussed in section III(D) of this Decision below) is cause for concluding that the approved uses of DES have not been shown to be safe and have been shown not to be safe.

(f) *Conclusion As to Radiotracer Studies.* I recognize that application of the results of the radiotracer studies to approved (and actual) conditions of use involves, in some cases, extrapolation. Such extrapolation is commonplace in science and is valid here. For the reasons stated above, I find that the radioisotope evidence discussed above demonstrates that approved (and actual) animal drug uses of DES, in sheep as well as cattle, will result in DES residues in edible tissues.

(3) *Findings by Department of Agriculture Monitoring Program.*—(a) *Evidence of Residues.* The Bureaus rely upon evidence that DES residues have been discovered in animal tissue by the Department of Agriculture as part of its monitoring program.

Dr. John Spaulding, Chief of the Residue Evaluation and Planning Staff of the Department of Agriculture, testified concerning that Department's residue monitoring program. He stated that steer and heifer livers are selected at slaughterhouses by inspectors in accordance with (1) a random sampling technique (described in some detail by Dr. Levy (G–58)) and (2) a number of sampling procedures designed to follow up on evidence of potential violations with particular lots of meat (Tr. at 470–71).

A portion of the liver is shipped to a laboratory where it is analyzed by the gas chromatography method. This method can detect (but apparently not positively identify) DES at levels as low as 0.5 ppb (Tr. at 492–93). If the gas chromatography analysis is negative, the liver is considered to be free of DES residues (G–94 at 2). If the analysis is positive, the entire liver is then requested and a second analysis is performed, again using gas chromatography procedures (id.). If this test does not confirm the first result, the liver is not recorded as having been shown to contain DES residues (id.).

If the second gas chromatography analysis is positive, a third test is run (id.). If the level observed in the first two tests is high enough, this reconfirmation will be performed by mass spectrophotometric analysis (id.). This procedure can detect levels of approximately 2 ppb (id.). If the first two gas chromatography tests had detected DES at a level lower than 2 ppb, the gas chromatography procedure is performed yet a third time using a different derivative of DES (id. at 2–3).

A liver found to contain DES by one or both of the first two gas chromatography procedures but not by the third test (whether it be mass spectrometry or gas chromatography) is recorded as a presumptive violation (see G–58 at 2–3). Dr. Spaulding noted correctly that the conservative policy of the Department of Agriculture (USDA) in requiring confirmation of the first gas chromatography test by the second may result in an understatement of the number of residues that actually occur (G–94 at 3).

The Bureau submitted the testimony of Dr. Bert Levy, a statistician from the Department of Agriculture (G–58). Dr. Levy stated the number of cattle and sheep slaughtered during the years 1971 through 1975, the number of cattle and sheep tested for residues from 1971 through 1976, and the number found to contain violative levels of DES residues from 1971 through 1976. (The total number of cattle and sheep slaughtered in 1976 was not available at the time the testimony was submitted.) Or the basis of these data he calculated, at a 95 percent confidence level, the percentage range (i.e., the lowest and highest possible percentage) of the total cattle and sheep slaughtered in that year that had violative DES residues. The numbers for cattle range from a low of 0.2–1.0 percent (reflecting 9 livers containing residues of an estimated 1780 tested) in 1976 to a high of 1.3–2.5 percent (reflecting 36 livers containing residues of 2003 tested) in 1972. Dr. Levy's calculations for sheep ranged from .09–0.6 percent in 1971 (5 livers containing residues of 1810 tested) to 0–3.7 in 1976 (0 livers containing residues of an estimated 100 livers tested).

Dr. Levy's calculations illustrate the fact that the number of DES residues found represents a much larger number of residues in the total treated population. If must be noted, however, that the percentages calculated depend more on the sample size than on the number of residues found. This fact is apparent from the calculations as to sheep stated above. In 1976, when 100 sheep livers were tested and no violations were found, the computed range of violations was 0–3.7 percent. This calculation is not intended to be evidence that the violation rate was as high as 3.7 percent. Indeed, as Dr. Levy's calculations show, the percentage of actual residues could be as low as zero.

The gas chromatography method of analysis was first supplemented by mass spectrometry in either 1974 or 1975 (compare Tr. at 496 (Dr. Spaulding) with Tr. at 725 (Dr. Levy)). Therefore, Dr. Levy's 1975 and 1976 figures (29 livers in 1975 equalling 0.8–2.7 percent violations and 9 livers in 1976 equalling 0.2–1.0 percent violations) were confirmed by mass spectrometry. The manufacturing parties emphasize that the gas chromatography method alone is not sufficient to identify residues positively as DES. This position is consistent with USDA's requirement of confirmation of gas chromatography findings by mass spectrometry in 1975 and 1976. In the table at the end of Dr. Levy's testimony (G–58), a number of apparent DES residues (15 in 1975 and 29 in 1976) are reported as presumptive violations, that is, violations that were found by at least one gas chromatography test but were not confirmed by "mass spectroscopy." (The term "mass spectroscopy" used in Dr. Levy's testimony is a synonym for the term "mass spectrometry" used elsewhere.) Because these residues have not been positively identified as DES, I place less weight on them than on the residues (stated above) that were confirmed by mass spectrometry.

As discussed above in the section on analytical methods, the gas chromatography/mass spectrometry method has not been shown to be sufficiently specific to serve as an analytical method for DES. Though this lack of specificity might make absolute confirmation of the residues as DES impossible, the USDA results are nevertheless probative evidence that DES residues exist in the tissues identified as containing DES residues by this method. An analytical method that does not meet all the requirements for routine regulatory use may nonetheless provide credible data for use in an evidentiary hearing.

The manufacturing parties argue that the Department of Agriculture findings of DES residues must be discounted due to three documents (M–18; M–19; G–28), which, they allege, show "apparent failures by Department of Agriculture employees to follow procedures that had been agreed upon with the FDA for the handling of samples of livers to be analyzed for residues of DES" (Manufacturing Parties' Exceptions at 49). The inference that the manufacturing parties seek to draw from these exhibits, i.e., that there was something wrong with the procedures utilized by USDA, is not warranted.

Two of the three memoranda reflect an agreement, reached in early 1974, to have USDA and FDA use the same method of gas chromatography analysis. An October 22, 1974, memorandum suggests that USDA had not made much progress in utilizing the agreed method (M–18 at 3). On April 18, 1975 (M–19),

the Bureau of Foods reported to the FDA's Associate Commissioner for Compliance that a new agreement had been worked out in accordance with which USDA would utilize the FDA method exclusively and then confirm by mass spectrometry the identity of any residues found. USDA would then report as positive any reading so confirmed. Neither of these memoranda shows that the procedures previously used by USDA were invalid, and thus neither provides a basis for disregarding the USDA residue findings.

The manufacturing parties take a sentence out of context from the third document referred to, a December 17, 1975, memorandum to the FDA's Chief Counsel from the Bureau of Foods (G-28), to imply that the FDA was not satisfied with the sampling technique utilized by USDA. In fact, the question raised there was whether USDA was cooperating correctly in a multi-laboratory test of the FDA's gas chromatography method. This document, also, provides no basis for disregarding the USDA residue findings.

It is apparent, therefore, that DES residues have been found in the past few years in the livers of cattle by the methods utilized by the Department of Agriculture's sampling program. (Although the majority of these residues appear to result from use of DES in feed, some result from the use of DES implants (Tr. at 769).) These residues have been identified in only a relatively small percentage of the animals tested, but it must be recalled that (1) not all residues will be caught by this system because the lowest level of measurement claimed is 0.5 ppb and (2) the residues found represent a significant amount of meat (1 percent of 25 million steers is 250,000).

(b) *The Question of Misuse.*—(i) *Necessity of Determining Whether Residues in Edible Tissues Result From Misuse.* The manufacturing parties argue: "The question * * * is whether the number of violations is so great as to show that the approved conditions of use are not 'reasonably certain to be followed in practice'" (Manufacturing Parties' Exceptions at 59). The question, however, is whether DES causes residues that have not been "shown to be safe," 21 U.S.C. 360b(e)(1)(B).

The manufacturing parties refer to 21 U.S.C. 360b(d)(2), which sets out factors that the Commissioner must consider in determining an animal drug's safety in the context of a *refusal to approve* an NADA. Because that section provides evidence of congressional intent with respect to the meaning of the term "safe," as used in the statute, it is appropriate to refer to it in a *withdrawal* proceeding as well. The section in question requires the Commissioner, in determining whether a drug is safe, to consider "among other relevant factors" four specified factors. One of these is "whether the conditions of use prescribed, recommended, or suggested in the proposed labeling are reasonably certain to be followed in practice," 21 U.S.C. 360b(d)(2)(D).

The manufacturing parties seem to argue that at some arbitrarily selected percentage of misuse of all animal drugs, "reasonable" misuse (to be tolerated) is divided from "unreasonable" misuse (to be the basis for a withdrawal). Then, they seem to argue, if residues are not found that prove that that percentage of misuse had been exceeded, the drug must be declared safe no matter how harmful the residues found may be to the consuming public.

This interpretation is inconsistent with the statute's terms. Whether conditions of use are reasonably certain to be followed is only one of several factors to be considered, and the ultimate issue is whether the animal drug is safe.

The term "reasonably certain to be followed in practice" must, in any case, be interpreted in the context in which it appears, i.e., as a consideration in deciding whether the use of a drug is safe. Thus, the amount of certainty that is reasonable necessarily varies with the danger posed by the drug. One degree of certainty would be required (i.e., reasonable) for a drug whose residue would kill a human consumer on the spot, whereas another degree of certainty would be required for a drug whose residue represented only a relatively remote danger to the ultimate human consumer. The failure to show the extent of the danger associated with residues of DES above 0.5 ppb (or above any level of residues—see section II(a) (2 and 3) of this Decision) prevents a determination that the reported residues are consistent with "reasonable" certainty that approved conditions of use will be followed in practice.

The manufacturing parties sought to introduce into evidence a document showing the extent of detected residues tolerated by the FDA for other animal drugs (M-148a). This document was properly excluded from the evidentiary record (see discussion of evidentiary rulings (section VI of this Decision) below). In any case, the argument that the percentage of residues detected for DES is no greater than the percentage of residues detected for other animal drugs is irrelevant. Because no safe dose for DES may be computed, DES cannot be compared to other animal drugs for which a safe dose can be computed.

Agency policy requires that the level of detection of the analytical method for an animal drug be set to pick up any residues above the safe dose for that drug. For carcinogens, a "virtually safe" dose or "no residue" level is utilized (G-24, see also 44 FR 17070; March 20, 1979). The percentage of detected residues for other animal drugs should, therefore, be the percentage above the safe dose. The percentage of residues computed for DES represents, at best, only the percentage of residues above 0.5 ppb, the lowest limit of detection of the gas chromatography method of analysis. We do not know how many residues occur above the "safe dose" of DES because no "safe dose" has been identified. Even if one accepts the Bureaus' witnesses' calculations of 1 ppt as a "virtually safe" dose, as I do not, no one knows how many residues occur above that level.

It is true that some animal drugs have been approved by the FDA using analytical methods that do not have a lowest limit of reliable measurement corresponding to a safe or "no residue" level by today's standards. Conceivably, some such NADA's may have been approved by mistake. Some are under review by the FDA now (see, e.g., 42 FR 43770; Aug. 30, 1977 (penicillin) and 42 FR 56254; Oct. 21, 1977 (chlortetracycline and oxytetracycline). (The cited documents are notices of opportunity for hearing in which one of the issues raised is whether the tolerance levels approved for those drugs are in fact, "safe levels.") The approval of other NADA's will be reviewed in an orderly manner in accordance with agency priorities pursuant to its ongoing "cyclical review" program (see 42 FR 64369; Dec. 23, 1977).

It may be that the FDA will find, after careful review, that it cannot determine the percentage of residues above a "safe level" or "no residue" level for these other animal drugs. If it makes that determination it will find, as I have done with respect to DES, that the existence of any amount of residues in edible tissues means that the approved conditions of use can not be found safe as "reasonably certain to be followed in practice." The comparison of the number of DES residues detected above 0.5 ppb with the number of residues detected for these other drugs is meaningless at this point.

I need not decide whether or not the residues found result from approved conditions of use. The residues present a safety question and (1) if they result from approved conditions of use, those conditions have not been shown to be safe or (2) if they result from misuse, then I can not find that the approved

conditions of use are reasonably certain to be followed, for the reasons discussed above. In either case, residues that have not been shown to be safe are entering the food supply in amounts that must be considered to pose a significant risk to the health of consumers.

(ii) *Evidence As to Causes of Residues.* I have, in any case, considered whether the record shows that the DES residues detected by USDA result either from following approved conditions of use or from misuse of the drug. The only evidence of potential value in resolving the issue are reports by FDA investigators. The Food and Drug Administration follows up on reports of DES residues made to it by USDA, in most cases by visiting the facility at which the animal was treated with DES. The Bureaus presented a set of approximately 140 establishment investigation reports ("EIR's") prepared by FDA inspectors who were seeking the cause of reported residues. This set of papers has been marked as Exhibit G–89. The Bureaus also presented a summary of EIR's from investigations of the causes of reported residues. That summary was marked as G–137.

The manufacturing parties note (Manufacturing Parties' Exceptions at 56) the discrepancy between the listing of the DES findings in Dr. Levy's testimony (G–58) and the DES residues noted in the summary of FDA investigations (G–137). For some years, G–137 lists more residues than does Levy; in other years, it lists fewer. The Bureaus have, however, explained this discrepancy: the FDA inspection figures are based upon not only the "objective" (i.e., random) sampling program described by Levy (see discussion above) but also the "for cause" program, which involves followup sampling of the products of previous offenders (Bureaus' Reply to Exceptions at 6). Thus, in those years when Levy reports more residues than the FDA, the FDA did not investigate each residue reported. Where the summary shows more residues, the FDA has investigated some residues found in the "for cause" program.

The manufacturing parties object to any reliance upon G–137 since the person who made up this summary was not presented for cross-examination. Some but not all of the EIR's summarized in G–137 were made part of the record as part of G–89. In reaching my decision I have relied exclusively on the EIR's actually made a part of the record in G–89.

The manufacturing parties suggest that only 12 of the 140 EIR's in G–89 do not show evidence of misuse (Manufacturing Parties' Brief, Appendix D at 1 n. *). Although my review of these EIR's reveals a somewhat larger number of EIR's lacking a showing of misuse, I cannot find that these reports demonstrate that DES residues occur when the approved conditions of use are followed.

Acceptance of the investigator's findings as evidence that residues will occur when the DES is used under approved conditions of use would reflect an unjustified confidence that where FDA inspectors had not found evidence of misuse there was no misuse. As misuse is a violation of the law, there would, of course, be incentive for feed lot operators to clean up before the FDA inspectors got to them. It would thus be surprising if FDA inspections caught the misuse in every instance. Therefore, I can not rely upon the relatively small percentage of investigations of residues that do not show misuse as proof that residues result when there is no misuse.

I conclude that the record does not permit resolution of the question whether the residues found by USDA are or are not the result of misuse of DES.

(c) *Conclusion As to Findings by USDA Monitoring Program.* The USDA reports demonstrate that residues in edible tissues do occur as the result of the use of DES pursuant to its approved (or actual) conditions of use, both in food and in implants, as an animal drug in cattle. The reports do not, due to the small number of tissues sampled in recent years, show whether or not use of DES as an animal drug results in DES residues in the edible tissues of sheep.

I conclude that it is not necessary to decide whether the residues found result from the approved conditions of use or from misuse of the drug. Whether or not the residues result from approved uses, the record demonstrates, as discussed in the sections on safety below, that these residues are potentially hazardous and have not been shown to be safe. To the extent that the possibility that DES will be misused is a factor in this safety decision, that factor does not support the safety of DES. The record provides no basis for a conclusion that the approved conditons of use are "reasonably" certain to be followed.

I have also made an alternative finding to obviate any need for remand in case a reviewing court should decide that I am obliged to determine whether or not observed residues result from misuse. That alternative finding is as follows:

(1) The observed residues result from misuse. Where the record does not contain sufficient evidence to decide a question, it is decided against the party with the burden of proof. As discussed above, the Bureaus have the burden of showing that residues are occurring under the approved conditions of use if a decision on that issue must be made at all. The Bureaus have failed in their burden, and the residues are therefore attributed to misuse.

(2) In light of the misuse demonstrated, I find that the approved conditions of use are not "reasonably" certain to be followed in practice.

(4) *GLC Residue Study.* Dr. Rumsey et al. performed one study of the fate of implanted DES in which radio-isotopes were not used (G–78).

Four lots of 16 steers were implanted with two 30 mg-DES implants each. Steers were sacrificed at 14 days, 28 days, 56 days, 84 days and 119 days. Animal tissues were analyzed, using identical gas chromatography techniques in two different laboratories. This test did not show the presence of DES in the tissues of animals slaughtered after more than 28 days. One of the two analytical laboratories found measurable DES in two of the animals slaughtered after 28 days but the other laboratory did not make that finding (G–76 at 2). The report of this study, and Dr. Rumsey, stated that the level of sensitivity of the gas chromatography method is 0.5 ppb (G–76 at 2; G–78 at 1). This study, as Dr. Rumsey stated (G–76 at 2), neither proves nor disproves that DES residues appear in tissues at levels below 0.5 ppb when DES implants are used in accordance with their approved conditions of use.

Part of the DES implants (about 20 percent of the initial weight) remained in the steers 119 days after implantation (G–76 at 2–3). This fact suggests that at least some DES implants remain in animals, releasing DES to their systems, 120 days after implantation. This finding supports my conclusion that approved conditions of use of DES implants result in residues in the tissues of the animals at slaughter.

(C) The DES Conjugates Issue

The Court in *Hess & Clark* stated as one issue to be considered in the DES hearing: "[W]hether the detected residues are composed solely of DES conjugates, and whether that substance is harmful; * * *", 495 F.2d at 994. The context indicates that the adverb "solely" refers to the manufacturers' arguments that the residues detected are solely DES conjugates as opposed to DES itself, and that the harmfulness of DES conjugates had not been put in issue.

Conjugates of DES are, according to the Bureaus' Dr. Kenneth Williams, "compounds composed of DES,

chemically linked to another molecule or molecules through one of its hydroxyl groups in such a fashion that hydrolytic [chemical or enzymatic] procedures may regenerate the parent compound" (G–99 at 2). Dr. Williams stated further: "In DES conjugates, the DES molecule is attached to another molecule but is otherwise structurally unaltered" (id.).

The manufacturers' Dr. Sieck stated under cross-examination that a test on which he was working had identified as conjugates of DES, the following: sulfate of DES, the monoglucuronide of DES, the monoglucuronide of methoxy DES and two other uncharacterized glucuronide conjugates (Tr. at 1370). Dr. Kaltenbach, another expert supporting the manufacturers' interests, stated that not all residues had been identified (Tr. at 2087).

(1) *Burden of Proof on Residue Issue.* The Court did not state who would have the burden of showing whether residues found are solely DES conjugates and whether those conjugates are harmful. It did make clear its rejection of the FDA's argument that a new discovery of unidentified residues is itself sufficient to show that an animal drug is no longer shown to be safe. The Court stated that the agency "has an initial burden of coming forward with *some* evidence of the relationship between the residue and safety to warrant shifting to the manufacturer the burden of showing safety. This is at least the case where, as here, the residues are of unknown composition" 495 F.2d at 993 (emphasis added); see also *Chemetron, supra,* 495 F.2d at 1000.

The question of what happens when new evidence shows that an approved animal drug adds unidentified residues to the human food supply is one of great importance to the FDA's ability to deal not only with DES, but also with other animal drugs. Chemicals such as animal drugs invariably are metabolized, at least in part, into other substances in an animal (or human) body. It is for this reason that the FDA requires identification of the principal metabolites of an animal drug, and demands toxicity testing and analytical methods for those metabolites, before it will approve an NADA (cf. G–24; 44 FR 17081 et seq. (March 20, 1979)). The agency's concern about these substances "formed in or on food because of the use of" the animal drug is in accord with the statute's requirements, 21 U.S.C. 360b(d)(2)(A).

Once an NADA is approved, as discussed previously, the agency can withdraw approval if "new evidence * * * shows that such drug is not shown to be safe," 21 U.S.C. 360b(e)(1)(B). Where new evidence shows that use of the drug results in residues of unidentified substances, the Commissioner must decide whether, despite his lack of knowledge of these substances, the drug may be considered to be "shown to be safe."

I reject the contention that the Court in *Hess & Clark* was demanding that the FDA identify the DES residues found and demonstrate that those residues are not safe. Such a requirement would place the public in danger during the period (perhaps years) necessary to characterize and test suspect residues of approved drugs. It would also put the FDA in the business of drug testing, a task that Congress intended to be the responsibility of the manufacturers of regulated products (see, e.g., H. R. Rept. No. 2284, 85th Cong., 2d Sess. 1 (1958)).

As noted, the Court in *Hess & Clark* did require "some evidence" of a link between the residue and safety before any burden is placed upon the applicant to identify observed residues and show their (and, thus, the approved drug's) safety. This requirement, not evident from the statute, is nevertheless met here. Those residues resulting from the use of DES that have been identified have been identified as DES and/or its conjugates (see, e.g., G–99 at 5–6; see also discussion above in section II (B) and discussion below). It is elementary biochemistry that the conjugation of a molecule, although it may change that molecule's activity quantitatively, rarely eliminates it.

This change in but failure to eliminate the activity of DES has been shown to occur with respect to the estrogenic activity of one conjugate of DES (see M–110 at 3; G–102: Comments on Vineland Laboratories Submission at 1; see also discussion in section III (c)(2) of this Decision below). Also, as discussed in detail below, DES conjugates would be expected to hydrolyze (break down) in the human body to form free DES, thus making DES conjugates as dangerous as DES itself. Therefore, there is substantial evidence in the record that warrants an inference that the DES conjugates are active in a manner similar to that of DES itself. Due to the recognized dangers associated with DES (see the discussion of the safety data with respect to DES below), there is, therefore, without question "some evidence" that residues identified as DES and/or its conjugates are unsafe.

Thus, if some evidence of a relationship between the residues found and safety is necessary, that evidence is present here. The manufacturing parties therefore have the burden of identifying the residues and showing them to be safe.

(2) *Failure of Manufacturing Parties to Satisfy Burden of Proof.* It is clear that the manufacturing parties have shown neither that the residues found are solely DES conjugates (rather than totally or partially DES itself), nor that DES conjugates are safe.

The manufacturing parties presented no data to show that all DES residues found would be in the conjugate form. They have not even advanced a theoretical basis that justifies an expectation that all residues would be conjugated.

The only investigation made of any of the residues detected to determine whether or not they contained free DES showed that in fact free DES residues were present, see G–103 at Tables V, VII, IX, X, XII, and handwritten tables. The Bureaus' expert witnesses did not rely upon this finding, however, and, as discussed below, the analyst who detected free DES noted that it can not be proven that the free DES he observed did not arise from hydrolysis of a DES conjugate during analysis (G–212: Comments on the Vineland Laboratories Submission at 1). I am thus left with a record devoid of support either for the proposition that the residues found are "solely" DES conjugates or for the converse of that proposition. The manufacturing parties have thus failed in their burden of proof on this issue.

Even assuming that all the residues discovered were DES conjugates, the manufacturing parties have failed to show that DES conjugates are safe. The only evidence in the record on this question is Dr. Kilman's testimony that DES-monoglucuronide had not caused renal (kidney) tumors in hamsters after 15 months (M–110 at 4 M–25) though it apparently did cause dysplastic changes in those animals (Tr. at 1827–28). (Cf. M–113 at 764 in which researchers suggest that it is a conjugated form of DES that is responsible for kidney tumors in hamsters.) The test cited by Dr. Kliman, of one animal species, for less than the animals' lifetime, in which the investigators looked only for one type of tumor, can hardly be accepted as evidence that DES conjugates are shown to be safe in man. It is perhaps noteworthy that the DES-monoglucuronide was administered subcutaneously in the hamster experiment (M–25 at 1252), a route that would be expected to prevent the metabolism of the glucuronide to DES itself (id. at 1255; M–110 at 3). As discussed below, the record provides evidence that DES conjugates are unsafe because they hydrolyze in the human body to DES itself.

Dr. Kliman also testified (M–110 at 3) that DES-monoglucuronide, when

administered by the subcutaneous route, had been shown in one test (M–111) to have 6 percent of the estrogenic potency (measured by effects on the cells of the vagina) of DES itself in rats and in another study to have 9 percent of the estrogenic potency (measured by effect on the weights of uteri) of DES in rats and 16 percent in mice (M–24). Dr. Kliman neglected to mention that the latter test showed that, when administered orally, DES-monoglucuronide had 40 percent of the estrogenic activity of DES in rats and 28 percent in mice (id. at 651). If one were to accept the manufacturing parties' argument that estrogenic activity is associated with carcinogenicity and toxicity, the evidence cited by Dr. Kliman in fact might be taken as some evidence that DES-monoglucuronide is unsafe. In any case, these data do not show the safety of DES conjugates.

Thus, I find (1) that the Bureaus have presented enough evidence (see subsection 1 of this section above) to raise substantial questions about the safety of the residues of DES; (2) that these residues consist of free DES or its conjugates or combinations of free DES and its conjugates; (3) that the manufacturing parties have not shown that the residues detected are solely DES conjugates; (4) that the manufacturing parties have not shown that DES conjugates are safe; and (5) that therefore the safety questions raised by the Bureaus remain unresolved. These findings, together with my finding (discussed above) that new evidence has shown that use of DES as an animal drug produces residues in edible tissues of treated animals, constitute a sufficient basis for withdrawal of approval of the DES NADA's.

(3) *Findings Assuming That Bureaus Have Burden of Proof.* The manufacturing parties read the Court in *Hess & Clark* and *Chemetron* as assigning to the Bureaus "the burden of coming forward with evidence sufficient to resolve * * * in their favor" the issues of the identity of the residues found and whether those residues are harmful (Manufacturing Parties' Exceptions at 70–71). I now consider the evidence in the record under this standard.

(a) *Evidence That Residues Contain Free DES.* Dr. Williams analyzed the livers of steers implanted by Dr. Rumsey et al. with radioactive DES (see, generally, G–99). (These radio-isotope studies are discussed in detail in section III(B)(2) of this decision.) Dr. Williams sought to determine whether any of the radioactive residues that were found in the livers of the treated steers were in fact free DES. He found free DES. (G–103 at Tables V, VII, IX, X, XII, and handwritten tables G–102: Comments on the Vineland Laboratories Submission at 1).

The manufacturing parties take the position that no free DES was actually found by Dr. Williams (Manufacturing Parties' Exceptions at 75–76). They focus on Dr. Williams' analyses of residues found in the liver samples from the two steers implanted with radioactive DES that were slaughtered after 120 days.

The attack on the findings in the first of these two liver samples is premised upon a mischaracterization of Dr. Williams' testimony on cross-examination. The manufacturing parties state, incorrectly, that Dr. Williams conceded that the amount of radioactivity detected in the "free fraction" of this first sample was so close to background radiation as to make his finding of free DES meaningless (id.). It is important to note, however, that Dr. Williams analyzed for free DES three separate subsamples of each sample of liver provided by Dr. Rumsey (see, e.g., G–103 at Table VII). At the hearing, Dr. Williams was asked about the subsample in which the radioactivity of the fraction of the residue identified as free DES was the lowest. He stated that the accorded no particular significance to the results for that subsample because they were so close to background (Tr. at 702). The manufacturing parties rely on this comment by Dr. Williams. The comment applies only to one of the three subsamples analyzed from the liver samples from the first 120-day steer. The fact that each of the three subsamples of the first liver sample produced a result above background provides more assurance that the result was a true one than would a single subsample standing alone. In addition, each of the other two subsamples of this first liver sample produced a result higher than the one about which Dr. Williams was questioned. Dr. Williams stated that he thought his findings for this whole sample (and the sample from the second 120-day steer) were significant (G–102: Comments on Vineland Laboratories Submission at 1).

The liver sample from the second 120-day steer produced slightly higher findings of free DES than the sample from the first steer. The manufacturing parties also attack Dr. Williams' findings with respect to the sample from the second 120-day steer, in part by taking out of context statements made by Dr. Williams.

"Counts per minute" are the units of measurement of the method by which Dr. Williams analyzed the residue. In the liver sample from the second 120-day steer, Dr. Williams observed free DES that provided a response of about 2 counts per minute above the background rate (Tr. at 702). The manufacturing parties rely upon statements by Dr. Williams dealing with his analysis of a different part of the residue (the hexane fraction) found in the livers (Manufacturing Parties' Exceptions at 76). He stated that "for these particular samples" (i.e., the samples tested in the haxane fraction analyses) 2 or 3 counts per minute would be "on a shaky line" (Tr. at 684) and elsewhere stated that 2.1 cpm would be "marginal above background" in the hexane analyses (Tr. at 691). While these statements were equivocal, I take them to mean that, *for the analysis of the hexane fraction,* 2–3 counts per minute was too low to produce a reliable result. Dr. Williams does not seem to have admitted, as the manufacturing parties suggest, that his findings in *his analysis for free DES* with the second 120-day steer were insignificant. In fact, he stated unequivocally that these results were not as was suggested to him during cross-examination, "meaningless" (Tr. at 702).

The manufacturing parties state that the Bureaus' Dr. Aschbacher testified that it was necessary to detect counts per minute of more than twice the background rate (not found for the two 120-day steers) in order to have meaningful results (Manufacturing Parties' Exceptions at 77). Yet the transcript reference cited makes it clear that Dr. Aschbacher's conclusion was applicable only to his own study, because of that study's design (Tr. at 597–98).

The manufacturing parties' witness Dr. Tennant stated his opinion that the low number of counts per minute observed in the residues found in the livers of the two 120-day steers were "marginal" (M–132 at 16). (Manufacturing parties' Drs. Lieberman and Kliman also made conclusory statements about the validity of the results observed with the 120-day steer livers (M–122 at 2, M–110 at 2).) The record shows, however, that Dr. Williams minimized the likelihood of error in his analysis by utilizing a relatively long counting time (Tr. at 684). I accept Dr. Williams' analysis of his own results.

The manufacturing parties argue that it has not been proven that an unidentified impurity was not responsible for the free DES observed Manufacturing Parties' Exceptions at 77–78). My conclusion that Dr. Williams'

results are not totally attributable to the impurity called pseudo-DES is discussed above in section III(B)(2)(c) of this Decision. There is no reason to believe that significant impurities other than pseudo-DES existed in the radio-labeled DES or that, if they existed, they would have caused the tests to reveal free DES erroneously. Thus, this speculation does not provide a basis for discounting Dr. Williams's observations.

The manufacturing parties do not attack Dr. Williams' finding of free DES at much higher levels in the ^{14}C-DES residues found in the tissues of steers slaughtered less than 120 days after implantation with DES (see G–103, Table VII.) They provide no explanation—and I am aware of none—for why free DES would be part of the ^{14}C-DES residues in animals slaughtered at less than 120 days but would not be part of residues found at 120 days (cf. Tr. at 2122). The results found with the sub-120-day samples thus confirm the results seen by Dr. Williams with the 120-day samples.

Although the results of Dr. Williams' analysis of livers from animals fed DES (as opposed to those implanted with DES) were not discussed, Dr. Williams' tables reveal that he also found free DES in the livers from the steers fed radio-labeled DES (G–103: handwritten tables). The manufacturing parties have suggested no reason why, in any case, the evidence on this subject from DES implants would not be applicable to DES used in feed.

I find therefore that Dr. Williams' analysis revealed free DES. This finding, however, does not necessarily mean that it has been demonstrated that use of DES as an animal drug results in residues that contain free DES.

According to the analyst, Dr. Williams, it can not "be proven that the free DES did not arise from hydrolysis of some conjugate (other than monoglucuronide) during the work-up of the samples" (G–102: Comments on the Vineland Laboratories Submission at 1). (Dr. Williams added tritium-labeled DES-monoglucuronide to some of the DES tested. His parenthetical exclusion apparently was meant to make clear that the free DES did not come from hydrolysis of the added product.) Dr. Williams' analysis thus shows that the residues contain either free DES or a conjugate hydrolyzable to free DES.

As I found in section III(B) of this Decision dealing with the detection of DES residues, the record shows that use of DES as an animal drug results in residues, in the edible tisues of treated animals, of DES and-or its conjugates. As discussed above, there is no reason to believe that these residues would be "solely" DES conjugates as opposed to DES itself. Based on the evidence in the record, however, I cannot exclude that possibility. I thus consider the question whether DES conjugates have been shown to be unsafe.

(b) *Evidence of Lack of Safety of DES Residues.* I find, on the basis of evidence in the record, that if the DES residues in the edible tissues of treated animals are conjugates of DES, those conjugates would be expected to break down (hydrolyze) in the human body to DES itself. Evidence in the record that DES is unsafe, therefore, is equally applicable to residues of DES conjugates.

The finding that the residues found, if they consist of DES conjugates to the exclusion of free DES, would nevertheless hydrolyze in the human body to free DES is supported by the testimony of expert witnesses. Bureaus' witness Dr. Williams stated: "I feel that it is most probable that conjugated DES, occurring in animal tissues, will give rise to free DES after ingestion by humans". (G–102: Comments on the Vineland Laboratories Submission at 2). Manufacturing parties' witness Dr. Liberman made clear his opinion that whatever DES conjugates were found in the radio-tracer studies would be hydrolyzable by enzymes to free DES (Tr. at 2123–24).

Evidence in the record that supports these opinions includes (1) studies (discussed in the following paragraphs) showing that one conjugate, DES-monoglucuronide, hydrolyzes to DES (apparently in the digestive tracts) in human and animal bodies (G–96–98) and (2) the discovery of free DES, discussed above, in the radioisotope tests of DES. (Evidence in the record shows that the free DES found by Dr. Williams either was an actual free DES residue or was the result of hydrolysis of a conjugate of DES. My reliance on the Williams's data here assumes the latter explanation to be correct. The William's study may be taken as showing that DES conjugates are hydrolyzed to free-DES. It does not, however, prove that the conditions necessary for that hydrolysis occur in the human body.)

Studies showing that a conjugated form of DES, DES-monoglucuronide, will be transformed back to DES itself in human consumers were introduced by the Bureaus' witness Ms. Weissinger (G–95). These studies were done with rats in various stages of early development (G–96–97) and, in one case, with two human volunteers (G–97).

In the human study, two men were each administered simultaneously DES-monoglucuronide labeled with radioactive carbon and DES labeled with radioactive tritium. Their excretory products were then analyzed. The researchers found that the DES-monoglucuronide and the DES itself resulted in simular metabolic products in the urine of the volunteers. (The different radioactive labeling of the DES and the conjugate made it possible to trace the metabolites to their parent compound.) This finding, together with other indirect evidence, showed that the conjugate was hydrolyzed to DES in the intestinal tract prior to absorption into the bloodstream (see, generally, G–97.)

Ms. Weissinger concluded that the rat and human studies showed that diethylstilbestrol glucuronide is hydrolized in the intestine to produce free DES (G–95 at 2). Ms. Weissinger stated her opinion that the conversion of the conjugate to DES in the intestine is catalyzed by an enzyme known as Beta-glucuronidase, which is present in microorganisms normally found in animal and human intestines (id.).

Manufacturing parties' witness Dr. Kliman attached Ms. Weissinger's conclusions on several grounds. Chief among them is that the upper part of the human small intestine does not contain bacterial glucuronidase, which Dr. Kliman stated is essential to the hydrolysis of the conjugate (M–110 at 18, cf. Tr. at 850 (Weissinger cross-examination)). Dr. Kliman stated that absorption takes place in the upper part of the human small intestine (M–110 at 18). Therefore, he seems to argue, hydrolysis of the conjugated DES would not take place at a point in the digestive tract at which absorption of the freed DES could follow. The test showed, however, that DES metabolites traceable to hydrolysis of DES-monoglucuronide did appear in the urine of the human volunteers (G–97 at 601, 602). They could not have done so had there been no absorption.

Dr. Kliman also argued that the studies referred to by Ms. Weissinger must be discounted because the subjects (both humans and rats) were fasting, and introduction of the DES with food might affect the absorption or hydrolysis being considered (M–110 at 17–18). In the absence of data showing that the results of such a study would have been different under nonfasting conditions, however, this criticism provides no basis for discounting the results.

Dr. Kliman further criticized Ms. Weissinger's testimony concerning the study on two human volunteers (M–110 at 18). Dr. Kliman argued that there is no evidence to show whether the conjugate of DES was absorbed in the presence or absence of its glucuronide component (id.). He then stated that there was no demonstration of conversion of the

conjugate to DES in the intestinal tract (id.). Neither of these points addresses the issue, however, because the study did show, according to its authors, that DES and the conjugate of DES administered simultaneously resulted in the same metabolic products in the body (G–97). The report of the study states further:

> Since the ingested glucuronide conjugate was excreted as products other than DESG [the DES conjugate], it appears that conjugate hydrolysis occurs in the body. Hydrolysis of DESG to DES may be nearly complete, since similar amounts of sulfate conjugates and polar non-hydrolyzable metabolites were excreted in the urine after ingestion of DES and its glucuronide conjugate * * *.

(G–97 at 601). Thus, there is no need to determine whether the glucuronide portion of the conjugate was present during absorption from the intestinal tract and subsequently removed or was split from the DES molecule before absorption. The material fact is that the conjugate was hydrolyzed to DES within the human system.

Steers have been shown to conjugate DES to DES-monoglucuronide (as shown by the presence, in the urine of steers treated with ^{14}C-DES, of DES-monoglucuronide attributable to that ^{14}C-DES) (G–3 at 47–48). This evidence supports a finding that DES conjugates found in edible tissues of cattle and sheep include DES-monoglucuronide.

In any case, as discussed above in subsection (a) of this section, however, analysis of residues actually observed in the radiotracer studies revealed that those residues contain, if not free DES itself, then DES conjugates that hydrolyze to DES. That evidence suggests the likelihood that whatever conjugates do occur in animal tissues will be hydrolyzed to DES in the human body.

(c) *Conclusion As to Conjugates Issues Assuming Bureaus Have Burden of Proof.* For the reasons stated, I find that, if the Bureaus have the burden of showing that the residues found are harmful, they have carried that burden. The residues contain either free DES or DES conjugates that would hydrolyze to DES. Because DES conjugates hydrolyze in the human body to free DES, the questions raised about the safety of DES apply equally to the conjugates of DES.

(D) Evidence That DES Is Not Shown To Be Safe

(1) *Relationship of DES to Endogenous Estrogens.*—(a) *The Issues.* As discussed below, DES is not a natural estrogen. Yet, because DES has estrogenic effects, the manufacturing parties contend that it should be judged as if it were in fact a natural estrogen (Manufacturing Parties' Exceptions at 94 ff).

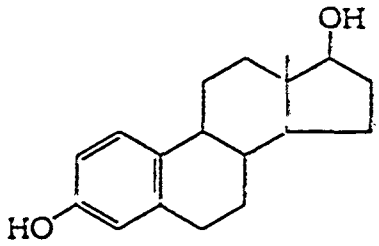
The manufacturing parties' theory is that the cancer and other adverse effects that natural estrogens cause occur only when those estrogens exceed the level at which they normally appear in the body (id. at 105–06). They argue further that the relatively small amount of DES added to the body through the ingestion (eating) of meat containing DES residues would not make the total level of estrogens in the body exceed normal levels (id. at 98–102), and that for that reason DES does not present a human cancer risk. It thus follows, they argue, that there is no danger in adding small amounts of DES to the human system (id. at 102 ff).

An assumption essential to the manufacturing parties' theory on this issue is that DES is simply another estrogen and that it has no carcinogenic or other adverse effects not associated with its estrogenic effects. The Bureaus dispute this assumption. They argue that there are significant differences between DES and natural estrogens and that DES may cause cancer and other adverse effects that would not result from natural estrogens at comparable dosages (Bureaus' Brief at 120 ff).

Manufacturing parties' witnesses seem to assume at the outset the proposition that they wish to support, i.e., that DES, which is not an endogenous estrogen, must be considered to be no different from an endogenous estrogen unless proven otherwise. They conclude, in effect, that because it has not been *shown* that all the adverse effects of DES are *not* associated with its estrogenic activity, it must be concluded that an association between DES estrogenicity and all of its adverse effects exists (see M–69 at 6 ("no compelling evidence" that tumor-enhancing properties not linked to estrogenic activity); M–110 at 6; M–62 at 5). Bureaus' witnesses, on the other hand, expressed the opinion that the lack of evidence that the adverse effects of DES *are* associated with its estrogenic activity prevents acceptance of that conclusion (see, e.g., G–80 at 8; Tr. at 164; G–90 at 6). Particularly in light of the demonstrated differences between DES and endogenous estrogens and the theoretically different ways in which the body deals with these substances (discussed below), I conclude that the record shows that DES cannot be considered as simply another estrogen.

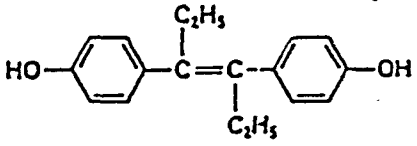
Even were DES "just another estrogen," it is by no means clear that it would be judged safe on that ground. The manufacturing parties agree (Manufacturing Parties' Exceptions at 97) that natural estrogens have been shown to cause cancer. See also Tr. at 1890; 2166–67. Estrogens have, in addition, been associated with other adverse effects (see, generally, 42 FR 37636, 37642 (July 22, 1977)). The fact that a dangerous substance occurs as a component of human tissues, cells, etc., (or is identical to a substance that so occurs) does not of itself justify approval of the addition of more of that substance to the human system by artificial means. *Cf.* I.D. at 35; *Bell* v. *Goddard, supra,* 366 F.2d at 182. Because DES can not legitimately be equated to endogenous estrogens, I do not reach the difficult question of how much (if any) of a substance chemically indistinguishable from endogenous estrogen could be added to the human body safely.

In discussing endogenous estrogens, the manufacturing parties refer most often to estradiol. Estradiol is a steroid (cf. G–189 at 2) that is produced by animals and man and is required for their proper functioning (cf. M–110 at 7). It influences biochemical physiological events associated with conception, birth, growth and development, and the proper functioning of adult individuals of the different species of mammals. The chemical structure of *beta*-estradiol (the most common form of estradiol) is as follows:

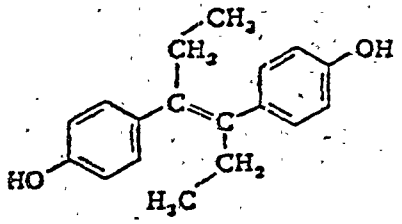


White et al., *Principles of Biochemistry* (5th Ed., 1973) at 1062.

DES is a stilbene (G–189 at 2; Tr. at 228). It is not produced by any species of animals, mammalian or otherwise, and is not required for the proper functioning of living organisms. It is produced synthetically. DES does, however, cause in mammals an array of physiological and toxicological effects that are remarkably similar to the effects produced by endogenous estrogens such as estradiol (and its metabolites, estriol and estone). DFS has the following chemical structure (G–47 at 419):



The manufacturing parties, while not disputing the validity of this rendition of the structure, proffer the following, which they apparently believe looks more like the structure of estrone given by the Administrative Law Judge (I.D. at 37 n. 23):



Manufacturing Parties Exemptions at 114, citing to Heftman & Mosettig, *Biochemistry of Steroids* (1960) at 167.

(b) *Differences Between DES and Natural Estrogens.* All parties agree that there are significant similarities between DES and endogenous estrogens. The hearing record establishes, however, that there are also incontrovertible differences in the chemical properties and in the biochemical and physiological effects of DES on the one hand, and estradiol (and other endogenous estrogens) on the other. For the reasons stated in the following discussion of these differences, I find, as did the Administrative Law Judge, that the observed differences bear on the toxicological significance of trace amounts of DES in meat from food animals.

(i) *Chemical and Biochemical Differences.* The Bureau's witnesses pointed to two areas in which the structural differences between DES and endogenous estrogens may lead to differences in effects. Each deals with the fate of DES and endogenous estrogens (specifically estradiol) within the body and raises unanswered questions about the claimed equivalence between DES and estradiol.

First, Bureaus' witnesses testified that there are differences in the way that the two substances bind to macromolecules in the body. These macromolecules, plasma proteins, attach themselves to smaller chemical molecules, such as those of estradiol and DES (G–191 at 2). Once bound, the molecules are hindered by the size of the macromolecule from leaving the circulation and reaching a target organ (id.) and, once there, entering the cell itself to do damage (Tr. at 73–74).

Although both estradiol and DES bind to the macromolecule albumin, estradiol, but not DES, binds to the much stronger binder, testosterone-estradiol-binding globulin (TeBG) (G–191 at 2). There is less TeBG than albumin in the body but TeBG binds so much more strongly to estradiol that its failure to bind DES must be considered significant. This is particularly the case because all active estrogens cause an increase in TeBG, i.e., the body protects itself from natural estrogens in a manner not available to counteract DES (id. at 3; G–90 at 6). Bureaus' witnesses point out that if significantly less DES than estradiol is prevented from reaching target cells, DES would be more dangerous than estradiol even if both had identical effects on the cell once they reached it (G–191 at 3; G–159 at 7).

It is noteworthy that this difference in binding resembles the effects observed in rats, though there it is alpha-fetoprotein rather than TeBG that causes the differential (G–159 at 2–7). Human alpha-fetoprotein binds well to neither estradiol nor DES (Tr. at 2309; M–203 at 5). Nevertheless, the analogy between rat experience with alpha-fetoprotein and human experience with TeBG, postulated by Dr. Sheehan (G–159 at 7), supports the question raised about differences in the human body's reactions to DES and estradiol.

The manufacturing parties' Dr. Jensen explained in proffered surrebuttal testimony his reasons for rejecting this theory. He stated that estradiol binding to TeBG is freely reversible, that albumin binds most estradiol, and that, even in pregnancy, TeBG binds only a relatively small fraction of the estradiol available (M–203 at 1–4). I explain in Part VI of this Decision dealing with evidentiary questions my reasons for agreeing with the Administrative Law Judge that Dr. Jensen's "surrebuttal" testimony was not proper surrebuttal and should not have been admitted. I have, nevertheless, considered his comments.

The record does not contain quantitative analysis of available data to support or reject either the theory that there are differences in the way DES and endogenous estrogens bind to macromolecules in the human body or Dr. Jensen's criticism of that theory. This potential difference between DES and estradiol, however, does raise an important question about the claim that the two substances are identical in their effects.

A second, less theoretical, area in which DES and estradiol are different is in the metabolites they produce. DES has been shown to yield, among other substances, dienestrol (3,4 bis (*p*-hydroxyphenyl)2, 4-hexadiene), *omega*-hydroxy dienestrol (3,4 bis (*p*-hydroxyphenyl)2-4-hexadiene-1-ol) (G–189 at 2–3; G–187 at 443) and *omega*-hydroxy DES (G–187 at 443; cf. G–189 at 3). Other substances, such as *para*-hydroxy-propiophenone, have been tentatively identified as metabolites (G–189 at 3). Bureaus' witness Dr. Helton testified that dienestrol and *omega*-hydroxy dienestrol are neither known nor expected to be metabolic products of any endogenous estrogen (G–189 at 3–4). No known metabolites of endogenous estrogens are similar to these substances in terms of structure or anticipated reactivity (cf. id.) This record does not provide a basis for determining whether the metabolic products unique to DES are the causes of some or all of the toxicity and carcinogenicity associated with DES (cf. M–203 at 5). I cannot discount the possibility that DES's metabolites exert effects that would not be associated with estrogens and their metabolites.

As the Administrative Law Judge noted, there is some evidence in the record that DES binds covalently to DNA (G–64 at 644) and is capable of damaging DNA (id. at 646). See also G–59 at 6. According to the manufacturing parties' own Dr. Jensen, such reactions are typical of chemical carcinogens foreign to the body or radiation, but are not typical of estrogenic hormones (M–69 at 6–7; see also Tr. at 2198; cf. G–59 at 6). Thus, the fact that DES and/or its metabolites is capable of binding with and damaging DNA is some evidence that DES may cause its carcinogenic effects (and other adverse effects such as teratogenicity and mutagenicity) by a mechanism that would not be expected of endogenous estrogens.

In their exceptions, the manufacturing parties attack the study that shows DES reactions with DNA. They argue that, of the two tests reported, one presented an artificial environment and the other produced only a relatively small effect (Manufacturing Parties' Exceptions at 122–23). The study that they contend involved an artificial environment does show that appropriately activated DES *can* react with DNA to modify it (G–64 at 644). The second study shows that this reaction does occur to some extent under more natural circumstances (id. at 646). These two studies do not provide unambiguous evidence that DES does indeed bind to and modify DNA. Yet the production by DES of reactions not expected to result from natural estrogens, like the production of metabolites not associated with natural estrogens, raises yet another unresolved question about the manufacturing

parties' assumption that DES is no different in its effects from endogenous estrogens.

(ii) *Physiological Differences.* The record establishes differences in the physiological (in this case, hormonal) effects of DES and those of estradiol. They are differences in the degree rather than the nature of the observed effects. For instance, the record shows the following: (1) Via the oral route, DES has about 10 times the estrogenic potency of estradiol (or of its metabolites estriol and estrone) (Tr. at 1784–5; cf. M–51 at 21, Table 3; cf. M–118 at 672 (20 times more effective in spayed mice)). (Estrogens cause cell proliferation and thus observable changes in the walls of the vagina. The potency of an estrogen is measured by, among other means, the extent of these changes.) (2) Intravenously administered estradiol is a more potent estrogen than DES administered via the same route in some species but not in others (M–110 at 9; see also M–115). (DES may be more potent relative to estradiol via the oral route than the intravenous route because by the oral route it is not oxidized (and thus neutralized) in the liver as estradiol is (cf. M–69 at 3).) (3) DES produces smaller changes in the vaginal mitotic index (changes in the rate of the multiplication of cells in the skin of the vagina) than does estradiol (M–40 at 4).

The differences in physiological effects between estradiol and DES shown by the record are of degree and not of nature. Endogenous estrogens may themselves differ in the strength of their physiological effects. Thus, the differences in physiological effects between DES and estradiol noted above would not be sufficient to reject the proposition that DES is no different from other estrogens.

Two points should be made about these data, however. First, the information in the record on the derivation of the comparisons noted above (see M–118) shows that they are based on effects observed at relatively high levels of DES and estradiol. These comparisons thus provide little usable information about the physiological effects, if any, of relatively small residues of DES in the edible products of animals treated with DES. Second, because of the differences in biochemical effects between estradiol and DES, I must reject the argument that these physiological effects of DES are necessarily related to its carcinogenic and other adverse effects.

I thus find that a comparison of the physiological effects of DES with those of estradiol (or other endogenous estrogens) neither supports nor detracts from the manufacturing parties' assumption that DES is equivalent to endogenous estrogens.

(c) *Conclusion As to Relationship of DES to Endogenous Estrogens.* In summary, the manufacturing parties have failed to demonstrate that DES is identical to estradiol (or any other endogenous estrogen) either in chemical structure or in biochemical or physiological (or toxicological) effects (cf. Tr. at 164–65; Tr. at 228–29). As Dr. Rosner stated, "There are differences [between DES and estradiol or other estrogens]. This is not the same compound" (Tr. at 2282; see also G–80 at 8; G–90 at 6). There are simply too many variables (and too many unknowns) inherent in the metabolic process and the processes leading to physiologic and toxicologic effects to conclude that DES is safe upon the basis of similarities to endogenous estrogens. In particular, the manufacturing parties have failed to establish that because the small amounts of DES introduced to the human body through residues in meat do not increase the body's level of estrogens DES presents no human cancer risk. On this record, I have no basis for concluding that the carcinogenicity of DES results entirely from its estrogenic activity.

(2) *Cancer Data.*—(a) *Animal Carcinogenicity Data.* DES is a carcinogen (G–22; G–34 at 1; G–37 at 2; G–46 at 2; G–47; G–59 at 2; G–70 at 2; G–80 at 7–8; G–84; G–85 at 6). This fact was stated unequivocally by one of the manufacturing parties' witnesses in a 1974 article that is part of this record (M–101 at 1920). This fact is also implicit in the analysis by the manufacturing parties of the results of the animal carcinogencity study conducted by Gass et al. (discussed below). (The manufacturing parties argue that, in that study, a carcinogenic response is observable in mice receiving 50 ppb DES and that that response increases with increasing dosage.) See also section I above.

Although the Bureaus submitted testimony to the effect that DES is a carcinogen in a variety of animals and NCI and IARC summaries of the studies showing that fact (G–47 and G–84), the only reports of animal carcinogenicity studies included in the record are the report of the Gass study and incomplete reports of an NCTR study.

(i) *The Gass Study. (a) Background.* The Gass study, entitled "Carcinogenic Dose-Response Curve to Oral Diethylstilbestrol" (G–22), appeared in the *Journal of the National Cancer Institute* in December of 1964. In this animal test, C3H female, C3H male and Strain A castrate male mice were divided into test groups that were given feed containing DES at the following levels: 0 ppb, 6.25 ppb, 12.5 ppb, 25 ppb, 50 ppb, 100 ppb, 500 ppb, and 1000 ppb. The test groups ranged from 50 to 78 mice. The three control groups ranged from 115 to 136 mice. The experiment was terminated after 85 weeks when the then surviving animals were destroyed in a fire.

A statistically significant incidence of mammary carcinoma was observed in the group of C3H female mice receiving the lowest dosage (6.25 ppb) of DES administered. The groups of C3H female mice receiving 12.5 ppb and 25 ppb did not show a statistically significant increase in tumors over controls. (Both of these treated groups showed tumors in 43.3 percent of the mice as opposed to 33 percent in the controls and 48.2 percent in the 6.25 ppb group.) There is no question that the C3H female mice fed 50, 500 and 1000 ppb DES developed mammary gland cancer and that the evidence of cancer in the treated groups increased with increasing levels of exposure.

The test groups of C3H male and Strain A castrate male mice were less sensitive. In each, some tumors developed in animals fed 12.5 ppb but statistical significance was not clearly apparent below the higher levels of exposure.

(b) Manufacturing Parties' Contentions. The manufacturing parties agree that this study (1) does not show that low levels of DES cause cancer and (2) does show that low levels of DES do *not* cause cancer, i.e., that there is a no-effect level (Manufacturing Parties' Exceptions at 126–27).

The first argument appears to assume that, if the only evidence that DES is carcinogenic was seen at dosages substantially above the levels of DES observed as residues, the FDA could not find that the levels observed as residues are unsafe or not shown to be safe. As discussed in the introduction to this Decision, however, the FDA must of necessity rely on tests showing effects of relatively high levels of a substance in test animals as a basis for the decision that lower levels of that substance present a carcinogenic risk to man. I have previously explained (in section III(D)(1) above) my reasons for rejecting the manufacturing parties' theory that the carcinogenicity of DES is related solely to its estrogenic activity. (If that theory were accepted, extrapolation from results of the ingestion of relatively high levels of DES in animals to predict the results of ingestion of lower levels of DES in humans might, of course, not be appropriate.)

In light of my rejection of the "carcinogenicity is a function of estrogenic activity" theory of the manufacturing parties, their second contention, that the animal studies show a no-effect level for DES, must also be rejected. Routine bioassays are not capable of establishing a no-effect level for a carcinogen. This proposition is well-supported by the opinions of noted cancer experts who testified at the hearing (G–46 at 8 (Dr. Hertz); Tr. at 172 (Dr. Saffiotti); Tr. at 1128 (Dr. Schneiderman); Tr. at 283 (Dr. Shimkin); *cf.* Tr. at 1176 (Dr. Herbst)). (The conflicting testimony of some manufacturing parties' witnesses is discussed below.) Thus, I can not find that the studies discussed in this section showed a no-effect level for DES's carcinogenic effect. This conclusion would stand even if the results of testing of DES at low levels were unambiguously negative. In fact, although the relative lack of sensitivity of the Gass study (G–22) makes interpretation of its results at low dose levels difficult, an apparent carcinogenic result was, as noted above, reported in that study at the lowest level tested (6.25 ppb).

Witnesses presented by the manufacturing parties supported those parties' contentions concerning the Gass study as follows: (1) Some witnesses gave their opinion that the lowest level of DES that cause a carcinogenic effect in the Gass study was a level (estimates varied as to what that level should be) above the lowest level of 6.25 ppb. (See, e.g., M–110 at 5; M–63.) (2) One witness testified that the results observed at the three lowest dosage levels of this study should be discarded because of the confounding effects of the fire that terminated the experiment (Tr. at 1948–51, 1969–70). (3) One witness testified that no valid statistical conclusions could be drawn from the study (M–139 at 8). My discussion of and evaluation of this testimony follows.

Neither the Bureaus nor the manufacturing parties called Dr. Gass as a witness. The manufacturing parties introduced an article authored by Gass and published in the Food, Drug and Cosmetic Law Journal (not a refereed scientific journal) in February of 1975. That article attacks the Delaney Clause. It comments upon Dr. Gass' own study as follows: "The lowest dose of DES that produces mammary cancer in the most susceptible animal species—the C3H mouse—required a minimum of 6.25 ppb—and probably four times that amount" (M–13 at 112). Elsewhere in the article Dr. Gass referred to the requirement of "at least" 6.25 ppb DES in a mouse diet to cause a carcinogenic effect and referred to the "probable carcinogenic dose level" of 25 ppb in the C3H mouse strain (id.).

Another manufacturing parties' exhibit (M–178) is a memorandum of conference between a Mr. Thomas Tomizawa and a Dr. R. L. Gillespie of the Bureau of Foods' Division of Toxicology. Dr. Gillespie, who apparently authored but did not sign the memorandum (dated March 23, 1976), quotes himself as having told Tomizawa "that currently Dr. Gass believed that 6.25 figure to be a biological fluke and that he believed the probability was that the true figure was somewhere between 25 and 50 ppb" (id.). The memorandum does not explain how Gillespie would know what Gass' then current beliefs were, and Dr. Gillespie was not called as a witness. Therefore the statement in the memorandum cannot be relied on.

No explanation is given by anyone as to why Dr. Gass was not called as a witness. Because the record reveals neither Dr. Gass' current views nor the basis for those views, and anyone disagreeing with them has not been given a chance to cross-examine him, I have accorded statements of his opinions less weight than those of witnesses who testified at the hearing. I cannot accept, without explanation, his apparent conclusion that some of the reported results of his study should be disregarded.

Manufacturing parties' witness Dr. Bernard Kliman explained his reasons for believing that the Gass study show that DES does not cause a carcinogenic effect at low levels (M–110 at 5):

> The log dose-response curve was linear only between 25 and 500 ppb. My further analysis of this data by extrapolation of this linear curve to intercept with the cancer incidence of the control animal group indicates no effect of DES on tumor incidence at or below 12.5 ppb.

Dr. Kliman disregarded the data points at the 6.25 and 12.5 ppb levels when fitting the probit-log dose line, and then noted that the observed responses at these two lower levels did not fall within the 95 percent confidence bounds of his extrapolated probit-log dose line (Tr. at 1832). It is not, of course, proper to exclude data from statistical analysis without evidence that those data are invalid.

Dr. Kliman, in dismissing the results at 6.25 ppb and 12.5 ppb, relied upon the fact that in the Gass study the lowest feeding concentration at which the weight of the ovaries was found to have decreased was 25 ppb. He stated: "It is reasonable to conclude that estrogens are associated with carcinogenesis only when given in amounts greater than the amounts required to produce a physiological response" (M–110 at 5). His only citation for this proposition was an article whose authors included Dr. Gass. This article contains basically that statement but provides no specific support for it. The article does state: "We should like to emphasize, however, that to the best of our knowledge, the relationship between the minimal physiological and minimal tumorigenic doses has not been determined for any of the estrogens" (M–64 at 23). (This article also contradicts the manufacturing parties' position on another point. In discussing the Gass study, it states: "As no levels below 6.25 ppb were fed, this study does not provide convincing evidence of a noncarcinogenic level in the C3H females," M–64 at 21.)

As discussed above, I have found that there is no basis for concluding that there is a direct relationship between the carcinogenicity of DES and its estrogenic effects. Thus, Dr. Kliman's exclusion of the results at 6.25 at 12.5 from his calculations makes his conclusions invalid.

The lead author of M–64, Dr. H. H. Cole, also testified for the manufacturing parties. Dr. Cole stated that physiological effects in the Gass study, i.e., ovarian weight depression, were noted at or about 13 ppb (M–62 at 3). (It is unclear where he got this figure.) He stated that 13 ppb would thus be the minimum level of DES required to cause a carcinogenic response (id.), although during cross-examination (Tr. at 1640) Dr. Cole admitted that at lower dosages there may have been physiological effects other than ovarian weight depression that went unnoticed. Dr. Cole did not state a clear factual basis for his hypothesis of a link between observed physiological effects and carcinogenesis. I cannot, therefore, accept that hypothesis.

Dr. Cole cited a paper by Jones and Grendon (M–63) for the proposition that the Gass study showed that the minimum carcinogenic level for DES is greater than 27 ppb. A review of M–63 reveals no such conclusion. The authors of M–63 do state that Gass reported that "DES induces mammary cancer in mice only at levels causing physiological distrubances, not lower levels," (id. at 264). M–63 then refers to tables in the Gass study without commenting upon the finding of a statistically significant effect at 6.25 ppb in the female test animals.

Dr. Hardin B. Jones testified for the manufacturing parties (M–97). During cross-examination, he stated a new theory to explain the finding of a statistically significant carcinogenic

effect in the 6.25 ppb group in the Gass study (Tr. at 1948–51, 1969–70). Because this testimony was introduced only on cross-examination, the Bureaus were denied a chance to prepare detailed cross-examination of it. I have, however, considered Dr. Jones' theory on its merits.

Dr. Jones relies, in this theory, upon the fact that the Gass study was terminated when a laboratory fire destroyed the remaining test animals (G–22 at 973). The study called for sacrifice of any animal in which a palpable, one centimeter, subcutaneous mass was found. After sacrifice, the mass was examined histologically. Those masses diagnosed as "mammary carcinoma" were designated as tumors in the results (id. at 972). Those animals destroyed in the fire were, of course, not examined for tumors. The Gass results consider these latter animals as having no tumors.

Dr. Jones argues that one should exclude from analysis all animals lost in the fire. Having done that, he finds that the results in the 6.25, 12.25, and 25 ppb groups are not different at a statistically significant level from each other.

This lack of statistical significance, however, could be due to the reduction in group numbers and the consequent reduction in statistical power to detect differences. Moreover, the results of Dr. Jones' analysis are, in any case, dependent upon the number of animals per group that exhibit non-cancerous subcutaneous masses. If a group had a relatively small number of animals with such masses, then the percentage of animals with mammary carcinoma would increase, and vice versa. (The report of this study does not provide information about how many, if any, mice died of natural causes before the fire.) Because it is not clear that noncancerous subcutaneous masses were a controlled variable in these groups (and no adjustments can be made for this fact), it is not appropriate to utilize the method that Dr. Jones has suggested to analyze the results of this test. If it were, as Dr. Jones suggested, improper to count all of the animals destroyed in the fire as not having tumors, then I probably would be best advised to disregard this study altogether. The weight of the expert evidence, however, including testimony for both sides in this hearing, suggests that the test results can be relied upon when properly analyzed. (See, e.g., M–110; M–62; G–21; G–25.)

Dr. Thomas Jukes testified that the Gass study showed a dose-response relationship starting at 25 ppb and that this relationship" with an absence of significantly larger numbers of tumors above controls below this level" showed a threshold (M–99 at 4). this comment, of course, ignores the result observed in the 6.25 ppb group. Dr. Jukes then stated that any reliance upon the results observed in the 6.25 ppb group separately from the results observed in the groups fed 12.5 and 25 ppb DES "defies biological common sense" (id. at 5). The Bureaus do not, however, ignore the 12.5 ppb and 25 ppb results (see discussion below). Relyng on any of these three results "separately" would, of course, be improper.

Dr. Jukes also stated that the "threshold" for tumor induction of DES in C3H mice "extends at least as far as 12.5 ppb and perhaps to 25 ppb" (id. at 6). This conclusion is based upon his report that the NCTR study, discussed below, showed fewer tumors in mice fed 10 ppb than in control mice. I explain below my reasons for not relying on preliminary reports of the NCTR data. Another, and more persuasive, analysis of the combined low dose results from the Gass and NCTR studies would be, however, that these studies are not sufficiently sensitive to show clearly any effect that might be associated with very low dosages. This interpretation is the conservative one and I adopt it. Therefore, these data do not provide a basis for the conclusion that a threshold has been shown for DES.

The manufacturing parties suggest that, because C3H female mice are highly susceptible to mammary tumors (in part because of the presence of a mammary tumor virus in that strain of mice), the results of test with this kind of mouse are not properly applicable to man (Manufacturing Parties' Exceptions at 136–138). The particular sensitivity of these mice, however, only makes tests with them more sensitive indicators of the carcinogenic effect of a substance such as DES. I cannot find that this enhanced sensitivity is reason for discarding test results achieved in female C3H mice.

The manufacturing parties also contend that this animal test is not equivalent to human exposure because in the animal tests the feed containing DES constituted the entire diet of the mice and that mice consume more food per unit of body than humans do (Manufacturing Parties' Exceptions at 137–38). These factors only make this test more sensitive to carcinogenic reactions. For the reasons discussed in the introduction to this Decision (section I(D)), it is necessary to use the most sensitive animal test system available in seeking information about the potential carcinogenic effects of substances such as DES.

The manufacturing parties' statistical expert, Dr. C. R. Weaver, raised questions about whether the environmental effects and the diet effects were completely separated in the Gass study (M–139 at 8–10). It is true that, if there exists "confounding" of effects, it is nearly impossible to distinguish statistically between them. Dr. Weaver's concern is that in the Gass study all the cages of animals receiving a particular diet may have been together (but separated from the cages of animals receiving other diets), and that therefore the different diet groups were subject to different environmental conditions (M–139 at 9). Dr. Weaver relied upon secondhand hearsay for some of his assertions (Tr. at 1518). I have evaluated his statements in that light and do not consider his testimony a proper basis for a finding that the Gass study did not have a satisfactory experimental design to avoid the confounding of the effects observed.

Dr. Weaver stated that all interpretations of the Gass study should be disregarded until further evidence is available (M–139 at 8):

> In view of the inadequate nature of the Gass data, the anomalous results obtained, and the suspect nature of the data at the lower end of the dose range, it is my opinion that statistical conclusions cannot properly be drawn form this study. * * *

Dr. Weaver's position, if accepted, would mean that the Gass study could not be used to establish a no-effect level for DES. He thus directly contradicts the testimony previously discussed.

(c) Bureaus' Contentions. The Bureaus' contentions with respect to the Gass study are straightforward. They argue that the study shows (1) that DES causes cancer in test animals and (2) that 6.25 ppb DES caused cancer in mice in that study (Bureaus' Brief at 39, 41).

As discussed above, even some manufacturing parties' witnesses based their testimony on the conclusion that the higher levels of DES fed in this study produced cancer (see, e.g., M–110 at 5). That proposition is not fairly open to dispute, and I agree with the Bureaus that DES at least at the 50, 100, 500, and 1000 ppb levels was shown to cause cancer in animals in the Gass study.

Testimony in support of the Bureaus' second argument emphasizes that the 6.25 ppb result in logically consistent with the results observed at 12.5 and 25 ppb and, in turn, consistent with the hypothesis that any amount of DES would cause some carcinogenic effect.

Dr. Robert J. Condon testified that he had investigated whether or not the probit-log dose model for the incidence rate of mammary cancer among the